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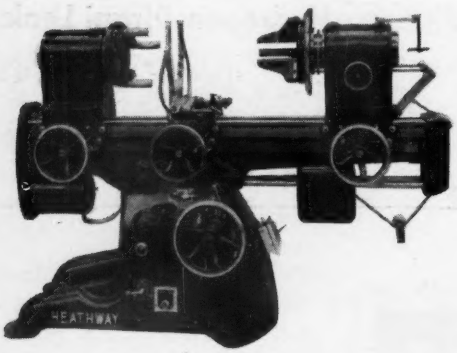

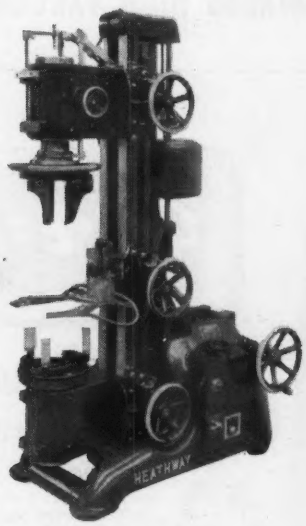

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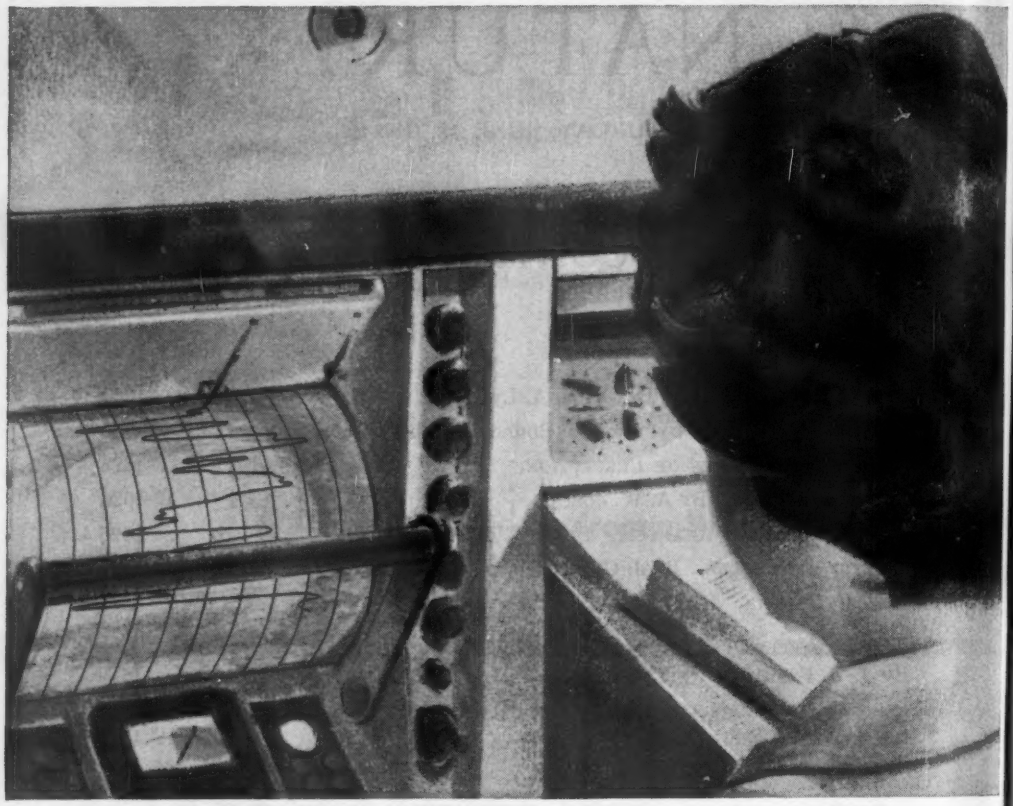

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TEACHING IN THE UNIVERSITIES

THE main functions of a university can be simply set down. There is the need to discover and interpret new knowledge; to impart knowledge and its interpretation to students; and to inspire them so that they will wish to join in the search for new truths themselves. In all that is done, the importance of imparting knowledge and understanding is pre-eminent. It is often wise to reflect how far this is successfully accomplished.

The university teacher has many critics; but those who are bad and beyond redemption are not nearly so many as the critics would have us believe. They are probably equal in number to those men and women who are naturally good lecturers and seem to have been born for the job. The large majority of university teachers are reasonably good craftsmen, sometimes aware of their deficiencies, conscious that their lectures frequently fail to impress, and whose shortcomings could be put right if they were prepared to spend some time learning their craft. They would get considerable help if they followed some of the advice put forward by G. Kitson Clark, reader in constitutional history in the University of Cambridge, and Commander E. Bidder Clark, who has had considerable experience with the use of visual aids to teaching in civil defence classes*.

As universities get bigger the problem of providing adequate and competent teaching staffs will become more and more acute. With increase in scientific and technological knowledge, the need for skilful exposition to promote real understanding will become more difficult, will require staffs prepared to make themselves good lecturers and who are prepared to use all the aids available to make their subjects intelligible.

The situation confronting a university lecturer is that confronting all lecturers. He must watch his audience to see that they are hearing and understanding him and that he is going neither too fast nor too slow. Yet how many lecturers find their inspiration on the ceiling, on the floor, or by the light of the windows: the students need not be present.

Nor should the lecturer be content with transmitting what is in his notes to the notebook of the student who, be he diligent, later returns them to the lecturer at an examination. The lecturer should seek to transmit what is in his mind to the mind of the student. This he will not do unless he periodically introduces checks to ascertain what is happening to the student's mind. A little acquaintance with the principles of pedagogy would help him considerably in practice.

For convenience, methods of instruction can be grouped into three. There is the straight lecture in which one voice alone is heard and which is particularly suitable for large classes. There is the discussion method in which a leader seeks to draw out and synthesize the combined knowledge and wisdom of a group. Then there is the 'lesson' method, so well developed in our schools, in which, by question and answer, the teacher uses an analytical method to develop knowledge and understanding in his pupil's mind. The tutorial class, so long an established feature at some universities, makes use of all three methods of instruction. The introduction of some questions and discussion would greatly improve the quality of many 'lectures', especially in science and technology, where the lecturer's voice is often broken only by the scratching of pens on paper.

Before a lecture begins, the teacher should consider his aims. As Kitson Clark and Bidder Clark write: he is there to serve his audience and should think of himself the whole time as "teaching *them*, interesting *them*, and giving *them* what they require, and can take". There is a limit to what men and women can digest in an hour, and too much produced too quickly in too complicated a fashion will leave them mystified and unsatisfied. The lecturer should aim at getting home a limited number of points, well defined, properly emphasized and arranged in some sort of logical order rather than in providing a running commentary or long stretches of narrative from which nothing stands out. Too often lecturers preparing their first course greatly exaggerate the amount that they will be able to cover in an hour as well as the amount of detail that they can deal with.

In general, the larger the class the slower the lecturer should go. The good lecturer, however, adapts himself to conditions which reveal themselves in the class-room itself.

The advice given by Kitson Clark is severely practical and contains hints as to how the lecturer should deliver his lecture. He suggests that the lecture should be divided into sections so that the lecturer may keep an eye on the clock and, if a section is taking up too much time, reduce it or cut it out altogether. The lecturer should take the class into his confidence and they should be told how he intends to arrange the course. A class which does not know where a particular lecture fits into a course will soon lose interest; and if, as some do, the lecturer conveys the impression that the course is not very important and rather uninteresting, he may not only lose the attention of the class but also their persons as well.

Many people find it a strain to listen too long to a lecture presented wholly in abstract terms. A lecture should be relieved from time to time by something concrete, a story used as illustration, a piece of narrative, or a striking fact which clinches some argument and makes it memorable; illustrations

* The Art of Lecturing: Some Practical Suggestions. By Dr. G. Kitson Clark and Commander E. Bidder Clark, O.B.E. Enlarged edition. Pp. 41. (Cambridge: W. Heffer and Sons, Ltd., 1959.) 3s. 6d. net.

should be subordinated to the general purpose of the lecture.

The successful lecturer never under-rates his hearers' knowledge or under-estimates their intelligence. He does not talk down to them—a matter of judgment and of sensitivity which grows from careful and continuous observation of their reactions during the course of a lecture. Above all, and this is probably the greatest fault with the majority of bad lecturers, he does not give the class at any time information which is readily obtainable from standard text-books. It is a sad commentary on university teaching that many students leave having read little more than the notes provided by their lecturers; the use of books as guides and friends is something they have to acquire much later.

The lecturer should consider his own physical position when he lectures. He should look at the class the whole time, and will be more effective if he stands rather than sits. If he stands he should always check his mileage; some lecturers travel surprising distances in an hour. (One famous 'walker' was estimated to have covered five miles during a striking lecture on the ketones.) Too much restlessness, manipulative tricks with one's gown, balancing on the edge of the rostrum, are disturbing and fidgeting to a class; the mouth should remain visible.

Some of Kitson Clark's most useful advice concerns voice production. Speaking at a wall from about 5 ft. off and cupping one's ears with one's hands gives a good idea of the effect on an audience. A lecturer should speak up with his head well up; shouting is less easy to hear than a comparatively soft voice with the words properly enunciated. A higher pitched voice is more easy to hear, but a lower pitched voice is more agreeable to listen to. As a lecturer becomes preoccupied or fatigued the voice tends to work up to a high note, sounding unpleasant and strained. This is more common in women than men; sometimes they inadvertently "sound aggrieved or lachrymose because their voices have got out of control".

It goes without saying that no lecture will be successfully presented unless it has been thoroughly prepared; one wit has argued that all good lectures are given the night before. Other matters which the lecturer should consider are the use to which he will put his notes, whether or not he expects his class to take notes, and the form they will take, and positive recommendations about books.

The case for the use of visual or aural aids to learning needs no arguing. Yet it is still surprising how little or badly they are used. The blackboard is a much-abused instrument which is seldom used to full advantage. Previous preparation of maps or diagrams in invisible (purple) chalk is of great help to the lecturer unskilled in cartography; a chalk broken before use saves many nerve-rending squeaks, especially if it is held at an angle of 45° to the board. Bidder Clark describes the contributions to better learning made by the blanket board, the prepared 'chart', models, the episcope and the film projector. These are well known but frequently little used because of the extra trouble involved in preparation and in manipulation during the lecture. The lecturer who is most concerned with teaching his class will undertake the additional preparation required and will always be welcomed by his class.

The fact that some university lecturers approach their teaching with the dilettante air of the amateur bewilders many students who have been brought up to expect high standards and find the change from school to university somewhat disturbing. They ask themselves why this should be so in institutions which are adorned by institutes and departments of education. Are university teachers unable to profit from courses on pedagogy? This is a matter which should be carefully considered by vice-chancellors and principals, particularly in the light of the Willis Jackson Report on the Supply and Training of Technical Teachers. The position could well arise that, in future, students taking Diploma in Technology courses at senior technical institutions may be better taught and acquire greater depth of understanding of their subjects than students taught by the all-too-prevalent lecturing method so widely used in British universities.

MAN AND THE UNIVERSE

The Individual and the Universe

By Prof. A. C. B. Lovell. (B.B.C. Reith Lectures, 1958.) Pp. vii+111. (London: Oxford University Press, 1959.) 10*s*. 6*d*. net.

ONLY those who have actually been confronted with the task of giving a series of broadcast lectures on such a subject as "The Universe" can fully appreciate the difficulty of the assignment: the Reith Lectures particularly so, for with these it is expected that the speaker will go beyond the mere science and say something about the social, political, and even religious implications of his subject. Moreover he is cut off from any easy reliance on the developed language of science, faced as he is by an audience spanning the whole population and ranging from mentally unskilled everymen to hypercritical scientists who tend to frown on such excursions by their colleagues into the popular realm. These latter may be right from some points of view, and it may well be useless pandering to the curiosity of the man in the street by telling him at his own level about the latest developments in science, but as with most lecturing it is the lecturer that profits most, and I imagine that Prof. Lovell will agree that such a public performance can be a thrilling and salutary exercise.

I have to confess that it is beyond my comprehension why it should be that the problem of the expansion of the universe should be singled out above other problems of science to become strongly associated not only in the popular mind but also in that of philosophers and even scientists with religious and ethical matters. Although the interior of the Earth is a good deal less accessible to direct observation than are the remoter parts of the universe, yet people would laugh if some well-meaning geophysicist were to lecture on "The Individual and the Inside of the Earth". But the Reith lecturer must deal with his audience as he finds it, and Prof. Lovell is to be congratulated on this masterly presentation of a

fascinating account of the epic struggles that scientists are now making to understand this mysterious universe. As effective owner of the world's largest telescope, Prof. Lovell naturally tends to lay emphasis on the importance of fabulously expensive equipment for solving these major problems, but unless strongly supported at every stage by theory, and the intelligent imagination needed to develop it, the meaning of the messages produced by such equipment will not always emerge. The encouragement of originality of thought, with the conflicts this involves, is perhaps the main social problem of science in our time or any other time so far, as Prof. Lovell is at pains to bring out in his talks.

The theories so far developed to account for the expansion of the universe, described herein, illustrate how easily theory can slip over into metaphysics. The 'big-bang' exploding super-atom hypothesis, with its attempt to 'explain' the expansion by postulating far less known things, seems to me reminiscent of Huygens' rarefied medium moving round the Earth with great velocity, introduced to 'explain' gravitation, when what is always wanted is a workable theoretical structure that can enable predictions to be made. My own feeling is that there is some major element still missing in our appreciation of this whole problem, and I would venture to predict that until some new theoretical approach on less mystical lines than hitherto is forthcoming, no serious progress with the problem will occur however large a telescope we or others build—mainly it seems at present, according to Prof. Lovell, to impress the neighbours. Nature seldom yields her secrets to this sort of wooing, but prefers the subtler approach.

I believe that the continued creation and maintenance of the universe will eventually seem as natural and intelligible by means of theory (yet to be established) as the view that the Earth is round seems natural and intelligible on gravitational theory, and that in such an attitude lies the only hope of solving this or indeed any other problem. Even though at present it seeems difficult compared with problems already solved, there are not the smallest grounds for suspecting that its solution is destined to remain for ever beyond the range of man's intelligence and inventiveness. And this spirit of hopefulness for scientific method is the main message that pervades Prof. Lovell's enthralling lectures.

R. A. LYTTLETON

CAMBRIDGE ANTHROPOLOGY

The Developmental Cycle in Domestic Groups

Edited by Dr. Jack Goody. (Cambridge Papers in Social Anthropology, No. 1.) Pp. vii+145. (Cambridge: At the University Press, 1958. Published for the Department of Archæology and Anthropology.) 21*s.* net.

THIS is the first in a projected series of symposia in social anthropology, each to be devoted to essays by Cambridge anthropologists on a particular theoretical topic. The theme of this issue is the changing form and significance of the domestic group during its development, and the analytical use of taking this process into account.

The volume comprises an introduction by Prof. Fortes and four papers, three of which are based on field research by their authors. Freeman, in a characteristically thorough ethnographic description, deals with the formation and partition of the family among the Iban of Borneo: his essay is of especial value in that they reckon descent cognatically, a widespread mode of relationship the theoretical interest of which has been obscured by an almost exclusive concern of anthropologists with lineal descent systems. Goody correlates differences in social groupings and processes of production with variant rules of inheritance in West Africa. Stenning relates the composition and stability of the pastoral Fulani domestic group to the herd on which it depends, and incidentally gives some information on a highly interesting dyadic system of symbolic classification. The concluding essay, not based on field research but an analysis of Trobriand data, is a brilliant demolition by Leach of Malinowski's assumption that the different and apparently conflicting meanings of the word *tabu* are unrelated homonyms. This theoretical exercise is related to the volume's theme mainly in its consideration of the significance of the change of a Trobriand youth's residence from his father's to his mother's brother's hamlet; but it is of far wider theoretical import than this theme, for Leach deals also with origin myths, incest and exogamy, political hierarchy, local groupings, land-holding, economic obligations, and why the Trobrianders happen to have four clans. It is a little chastening to reflect that such ingenuity as Leach displays is required to give systematic conviction to Malinowski's renowned data and to order the facts of Trobriand life as the people themselves do. This fascinating structural analysis shows how necessary and profitable it is to take native categories seriously, and how essential it is to understand the social structure if we are to grasp what the terms denoting these categories mean. The essay bears the marks of a sociological classic, and alone would justify the launching of a new publication. Its author is to edit and introduce the second number in the series—on caste—and one eagerly awaits this as any vehicle for the ideas of one of the most fertile and penetrating minds in social anthropology.

RODNEY NEEDHAM

EXPERIMENTAL STUDIES OF PERCEPTION

Readings in Perception

Selected and Edited by Prof. David C. Beardslee and Prof. Michael Wertheimer. (The University Series in Psychology.) Pp. xiii+751. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1958.) 66*s.*

THIS book is a valuable attempt to represent the present position in the experimental psychology of perception, and to some extent also the steps which have led up to that position, by means of outstanding papers and chapters from important books. The result is an extremely interesting and often rather bewildering collection of fifty-two selections grouped under five main headings.

The authors consider that they have selected studies which represent the core of the experimental psychology of perception, upon which so much current research in the fields of personality, motivation and social psychology is built. They have included a large number of recent investigations, which show the reader clearly what has been and is being done in the study of perception, and will certainly give him a vivid impression of the tremendous interest,

complexity and difficulty of the tasks of research. Some of the papers are models of clear thinking, lucid exposition and decisive conclusions. The instructive quality of others lies partly in the evident difficulty of satisfactorily controlling the experimental conditions, and/or drawing conclusions upon which confidence may be placed. Some papers are models of nearly unreadable prose, but in a collection in which the level of specialization of the contributions is unavoidably so varied, this may be inevitable. The editors have also wisely included some papers of classical interest, provided they have not been rendered obsolete by more recent research. These are landmarks for the reader. It is illuminating that in passing from the work of men of outstanding genius to that of ordinary experimenters there is often such a vivid impression of the differences between them. The problem of classification of the papers chosen seems to have been a very difficult one, and anybody reading the book or taking parts of it for discussions, lectures or seminars, would probably have to re-arrange the papers in an order which he felt to give a rational sequence.

The main themes covered by the book are: (1) biological factors in perception (two papers); (2) psychophysical measurement (one paper); (3) perception of objects and events, divided into five sections, (*a*) the stimulus field and its differentiation (four papers), (*b*) perception of organized figures and areas (twelve papers), (*c*) effects of learning and limited experience (four papers), (*d*) perception of successive and changing stimuli (seven papers), (*e*) space perception (eleven papers); (4) imagery and fantasy (three papers); (5) recognition and identification of figures, divided into two sections, (*a*) perception of words as stimuli (five papers) and (*b*) perceptual categorizing (three papers).

In reading the book it is interesting to consider whether the editors might not have done better to re-write the whole mass of material in their own words. At least the length of many papers could have been greatly reduced with advantage. Much boring matter could have been eliminated, and the essential points could have been brought into clear focus. However, in doing this, they might have lost the unique quality which belongs to the thought and writing of many of the gifted authors represented in the collection. R. W. Pickford

EARLY STEAMBOATS

The Birth of the Steamboat

By H. Philip Spratt. Pp. 149+11 plates. (London: Charles Griffin and Co., Ltd., 1958.) 28*s*. net.

THIS authoritative little book has clearly been a labour of love on the part of the author and it will be of interest mainly to the specialist rather than to a general reader.

The subject-matter is grouped under the three main headings of "Inventive Aspirations", "Experimental Steamboats" and "Steamboats in Service", and each chapter then deals in chronological order with the various inventors, experimenters or builders who had any effective impact on steamboat development. The book thus tends to be rather episodic, but the author attempts to overcome this by inserting cross-references in the text where necessary. There is a good index, and the list of references is impressive: 390 of them from libraries in Great Britain, the United States, France, Canada, Italy, Germany and Spain.

The material is given factually and without bias. The great names stand out and one cannot help but feel disappointment that the great French pioneer, the Marquis Claude de Jouffroy d'Abbans, was not successful with his fine vessel, *Charles-Philippe*; the ratchet mechanism that he used to drive the paddles proved defective. One of the most likely early vessels was one built by the partners, Fourness and Ashworth, of Yorkshire, having three steam cylinders acting on a three-throw crankshaft driving two paddle wheels. It is described as having undergone "severe tests on behalf of the Prince Regent". Unfortunately, the vessel was soon afterwards mischievously burnt before it had any chance of extended service.

The book clearly brings out the reasons for the failure or success of most of the vessels tried and, as well, it emphasizes that the success attained by that great American steamboat pioneer, Robert Fulton, was considerably due to his willingness to make himself familiar with the work of others in the use of steam and adapt their ideas to his own use. He made no claim to invention but concentrated his attention on well-tried mechanisms and tested hull proportions.

The book closes at 1822 with the development of the first iron-built steamer to put to sea, *Aaron Manby*, and the author has completed a volume which gives the background of the endeavour and the efforts, the triumphs and the failures of those pioneers who made modern ocean commerce possible.

In talking of the size of machinery, reference is often made to a figure for 'nominal horse-power'. It would have added to completeness if a definition of this term had been included. A. W. Jones

MODERN TAUBERIAN ANALYSIS

Tauberian Theorems

By Prof. H. R. Pitt. (Tata Institute of Fundamental Research Monographs on Mathematics and Physics, No. 2.) Pp. x+174. (London: Oxford University Press, 1958.) 37*s*. 6*d*. net.

THE integral transform:

$$f(x)=\int k(x,y)\, g(y)\, dy$$

where k is a given kernel, defines f, in a sense, as an average of g; hence we might expect that while properties of f will follow the type of properties of g, the converse inference will not usually be possible without supplementary conditions. Thus Abel's theorem, that if Σa_n is convergent, $\lim \Sigma a_n x^n$ exists as $x\to 1$, has a false converse, which becomes true if supplementary conditions are laid on the a's. Tauber (1897) gave the condition $na_n\to 0$, but this, though sufficient, is not necessary.

The first phase of Tauberian theory began in 1910, when in spite of formidable difficulties, Littlewood showed that a sufficient condition for the Abel converse is that na_n should be bounded; the distinction between tending to zero and being bounded marks a general and characteristic difference between the 'elementary' and the 'deep' theorems in this field. The Hardy–Littlewood partnership then obtained and exploited numerous Tauberian theorems. The second phase dates from about 1923, when Wiener showed that practically all the known Tauberian theorems could be expressed in terms of integral transforms with kernels of the type $k(x-y)$,

thus shifting emphasis from results about summation processes for divergent series to a more general theory of transforms. Since then the theory has branched out in several directions, and a comprehensive treatise would be voluminous. But along the main lines laid down by Hardy–Littlewood and Wiener, simplifications and generalizations have been made by many workers, among whom Prof. Pitt has been prominent.

He has now organized this material into a single short volume, the second in the series of monographs on advanced topics sponsored by the Tata Research Institute of Bombay. It is a book for the professional mathematician contemplating work in this field, and a fair degree of sophistication is needed to follow the clear but very concise reasoning. There are chapters on the Hardy–Littlewood and Wiener theorems, and one on theorems developed from Mercer's result (1907), that if σ_n is the mean of $s_0, \ldots, s_n$, and $\alpha > 0$, the convergence of $\alpha s_n + (1-\alpha)\sigma_n$ implies the convergence of s_n; this has the remarkable air of being a Tauberian theorem without a Tauberian condition. Finally, there is a chapter on the prime number theorem; here it appears essential to work with means related to the primes, so that Tauberian theorems are obviously relevant. But Pitt points out that neither the Ikehara-Landau proof, perhaps the simplest, nor the recent Selberg-Erdös proof, certainly the most elementary since it demands no knowledge of Riemann's ζ-function, uses Wiener-type Tauberian theorems, and he offers some pointers towards a study of general Tauberian theorems which are not of Wiener's type. If this prompts some young research worker to investigate the topic, the author and the Tata Institute should be gratified. T. A. A. BROADBENT

MAGNETIC AMPLIFIERS

The Theory and Design of Magnetic Amplifiers

By Dr. E. H. Frost-Smith. (Automation and Control Engineering Series.) Pp. xix+487. (London: Chapman and Hall, Ltd., 1958.) 75*s*. net.

THIS well-written and comprehensive book can be recommended to both students and professional engineers, and many will wish that it had appeared earlier.

The introductory chapter is noteworthy for the emphasis which it puts on mean rectified values of voltages and currents in terms of which transductor characteristics are conveniently described. Two chapters are then devoted to the principal parameters and characteristics, both steady-state and transient, of transductors with idealized core-materials. From this basis the theory of self-excited and auto-excited transductors with practical core-materials and imperfect rectifiers is developed and the influence of reactive loads is discussed.

A short chapter on the theory of control systems is included and a further treatment of the dynamic response of transductors with resistive and reactive loads is discussed in terms of control-system theory. This leads naturally to the need for quick-response transductors in control systems and an admirable chapter is devoted to half-wave, full-wave and three-phase transductors having short-response times.

The three chapters which follow deal with balanced magnetic amplifiers, low-level signal amplification and cascaded amplifiers, and magnetic modulators.

The section on design summarizes the properties of core-materials and their influence on transductor characteristics. A design procedure is developed and is illustrated by three examples, one of which extends to the overall performance of a magnetic-amplifier position-servo. The mixture of units with which engineers still have to deal, despite the adoption of the M.K.S. system for basic equations, is apparent in this chapter.

The final chapter on applications covers a wide field and leaves no doubt as to utility of transductors.

Most chapters have concluding summaries and these, together with the list of references to original papers, make this book a valuable acquisition for engineers entering this field. J. G. HENDERSON

TREES OF BRITAIN

Trees of Britain

By Dr. Robert Gurney. Pp. 228+18 plates. (London: Faber and Faber, Ltd., 1958.) 30*s*. net.

THE late Dr. Robert Gurney had wide biological interests and collected a great deal of information from his own painstaking observations over a period of many years, particularly around Oxford (his home) and in Norfolk. Trees were, perhaps, his primary interest in his later years, and an earlier book of his, "Our Trees and Woodlands" (Medici Society, 1947), will be known to many. The present volume possesses certain special features that are not to be found in the usual books dealing with trees in the British Isles. Most of the books that have appeared hitherto have dealt either with the native trees or with the introduced trees, grown for ornament or for timber. In the present volume Dr. Gurney deals with both these categories, covering all the common trees, wild or cultivated, that are to be found throughout the country. About one hundred different species are dealt with, each receiving separate treatment. The general information accompanying each description includes facts concerning the origin of each species, date of introduction to Britain (where known), general behaviour and growth characteristics. The clear line drawings that accompany each description are a notable feature of the book. They are the work of Dr. Gurney himself, and they usually include a drawing of the seedling, which the author raised from seed which he had himself collected. Another feature of the book is the emphasis that has been placed on buds or bud morphology, and bud development, the features brought to light being illustrated in the drawings. In addition to line drawings there are photographs showing individual trees or groups of trees in various parts of the British Isles. These are all pleasing or exceptional photographs of their kind and may well have been selected for their aesthetic value.

In the section on "Conifers and their Allies", all the species yielding softwoods of importance in Britain or of use in other respects are described. A table giving the dates of introduction of coniferous trees to Britain is of interest. From this it appears European spruce (*Picea abies*) is the earliest introduction (1548), while *Metasequoia glyplostroboides* is the most recent (1948). It is of interest to note that two important conifers, Japanese larch (*Larix leptolepis*: introduced 1861) and western hemlock (*Tsuga heterophylla*: introduced 1863), have not yet been in cultivation for a hundred years in the British Isles.

F. N. HOWES

How to Know Western Australian Wildflowers

By William E. Blackall and Dr. Brian J. Grieve. Part 2 : A Key to the Flora of the Temperate Regions of Western Australia. Pp. xxxiii+321–460+lvii. (Nedlands, W. A. : University of Western Australia Press, 1956.) 30*s*.

SINCE Gaston Bonnier and G. de Layens—with their classic "Flore de la France", published so long ago as 1887—introduced the illustrated-key type of flora, there have been many imitations. The book noticed here is the first example dealing with any part of the flora of Australia and is, in the reviewer's opinion, a good example of this type of flora. Actually the present work is Part 2 of a book first published in 1954, this part containing families 60 to 72 of the original scheme and also the family Goodeniaceae which is of especial interest in Western Australia. Dr. Blackall's original manuscripts have again been worked up by Dr. Grieve and the whole checked against as much fresh and dried material as could be obtained. From examination of the keys forming the flora it is clear that the results of monographic or other specialist work have been fully incorporated.

On the whole the drawings accompanying the different alternatives presented in the keys illustrate well the characters mentioned in the text, though in some cases the small scale makes it difficult to show the special features as clearly as one would have liked. This, however, is a common defect in such illustrated floras. Small sketches of all the species keyed-out help the student to confirm the correctness of his determinations. A complete key to the families in the whole flora is included, while the index to the species gives the authorities for the names and also the distribution according to a standard map provided. There is also a useful glossary. In addition to the many drawings in the key itself, there are five coloured plates which illustrate some of the more striking plants included in this part. Altogether, Dr. Grieve is to be congratulated on the continuation of a useful book. V. S. Summerhayes

The Cherry Kearton Animal Book

By Cherry Kearton. Pp. 168+16 plates. (London : Hutchinson and Co. (Publishers), Ltd., 1958.) 12*s*. 6*d*. net.

SOME of the best stories and anecdotes of Cherry Kearton's pet animals have been incorporated into this entertaining volume. In it the reader will meet Simba, the intrepid fox terrier, whose adventures in Africa are a far cry from his acquisition by Cherry Kearton at the Battersea Dogs' Home, and Toto, the intelligent and mischievous chimpanzee who accompanied his owner on some of his African travels. Another section of the book illustrates the wide range of Cherry Kearton's interest in animal life and recounts his friendships with a penguin, an Algerian sand rat and a trapdoor spider. The book closes with some stories of Mary, in her time probably one of the best known chimpanzees in the world, whose accomplishments seem at times to be almost human.

Cherry Kearton's pleasant and easy style carries the reader effortlessly through these stories of his animal friends, and throughout them there runs his understanding that patience and kindness are the only ways to gain the trust and affection of animals. The book is illustrated with photographs of its characters, and in addition there are a few photographs of animals in their natural setting. There is little to criticize : deer do not occur in Africa and three of the photographs have little relation to the text. The book has an obvious appeal to children, and is an admirable introduction for them (and perhaps for their parents) to the writings of Cherry Kearton.

J. E. Hill

Nuclear Reactor Metallurgy

By Walter D. Wilkinson and William F. Murphy. With a chapter by Warren J. McGonnagle. Pp. viii+382. (Princeton, N.J. : D. Van Nostrand Company, Inc. ; London : D. Van Nostrand Company, Ltd., 1958.) 42*s*.

THIS book is based on a course of lectures given at the Argonne National Laboratory since 1955. Appropriately, at least half of it deals with uranium, which is treated in detail from the ore to radiation damage in the reactor in a series of chapters. Other chapters deal with plutonium, thorium, ceramics, canning and control rod materials, liquid metals, and radiation damage in engineering materials. A final chapter on non-destructive testing is contributed by Warren J. McGonnagle. In general, the treatment of the chemical and metallurgical engineering topics is much better than that of the physical topics. The important effects of thermal and irradiation strains on the mechanical properties of uranium are overlooked, and the discussion of specific heats and thermal conductivity in Chapter 6 is very weak. Radiation damage in graphite is dismissed in four lines. This latter, as well as the omission of a discussion of magnesium as a canning material, is perhaps understandable since the authors confine themselves mainly to American reactors ; but it will disappoint British readers. The chapter on liquid metals is interesting, as are also those on thorium, beryllium, and zirconium, and the book as a whole serves as a useful partial account of the science of reactor materials. A. H. Cottrell

Truth and Denotation

A Study in Semantical Theory. By Prof. R. M. Martin. Pp. xii+304. (London : Routledge and Kegan Paul, Ltd., 1958.) 42*s*. net.

THIS is likely to be a useful book, both to the specialist and to anybody who is prepared to take some trouble to read and think carefully about formalized languages and their place in the methodology of science. It will go some way to answer the question as to why mathematicians and logicians invent the particular signs and symbols which are now becoming almost common coin. The first chapter deals in general with this theme, whereas the rest of the book is an able and clear discussion of the machinery, including such topics as first-order languages, syntax, non-translational semantics, and the theory of sets and types.

It is well to recollect that it is a feature of non-translational meta-languages that they are not strict translations of the object language. Thus is introduced the relation of 'comprehension'. The importance of this, among other things, is that the classical system of Aristotelean logic is not very satisfactory, when *relations* are involved ; but from here, with one semantical primitive, one can build up a species of extended Boolean algebra. It will be interesting to see whether it will stand up to the desire of some applied mathematicians to use these methods for complex electrical networks. Considerable success has already attended efforts to do so with some of the 'classical' Boolean equations. F. I. G. Rawlins

THE ROCKEFELLER INSTITUTE, NEW YORK: A NEW GRADUATE UNIVERSITY

By L. J. F. BRIMBLE

History and Constitution

IN the United States, it was considered that up to 1900 the growth of medical science had not kept pace with the physical sciences, despite the constantly increasing rate at which medical research had been carried on in American universities. Therefore, Mr. John D. Rockefeller accepted the expressed opinion of his adviser, Frederick T. Gates, that "medicine could hardly hope to become a science until it should be endowed, and qualified men be enabled to give themselves to uninterrupted study and investigation, on ample salary, entirely independent of practice". As a result, the Rockefeller Institute for Medical Research was founded in New York during 1901 by Mr. Rockefeller "to conduct, assist and encourage investigations in the sciences and arts of hygiene, medicine and surgery, and allied subjects, in the nature and causes of disease and the methods of its prevention and treatment, and to make knowledge relating to these various subjects available for the protection of the health of the public and the improved treatment of disease and injury . . . to use by any means . . . including research, publication, education, and the establishment and maintenance of charitable and benevolent activities, agencies and institutions appropriate thereto . . .".

The original Board of Directors, comprising William H. Welch (president), T. Mitchell Prudden, L. Emmett Holt, Theobald Smith, Christian A. Herter, Herman M. Biggs and Simon Flexner first set out to study the availability of adequately trained younger men in universities for the furtherance of medical research. The first rented buildings, at 127 East 50th Street, were directed by Simon Flexner, and there investigations in pathology, bacteriology, physiology and biological chemistry were conducted, thus immediately establishing medicine as a science. Later, Mr. Rockefeller purchased the land overlooking East River in York Avenue at 66th Street which now forms the present campus of the Institute. There he had built a laboratory, now known as Founder's Hall (Fig. 1) which was completed in 1906, and this provided space for experimental surgery and experimental biology in addition to the subjects already mentioned.

In 1908, Mr. Rockefeller provided further funds for the establishment of a hospital and isolation pavilion (Fig. 2). This was opened in 1910 with Rufus Cole as director. To this day, only patients of special scientific and medical interest are admitted on the recommendation of a panel of medical men. There they receive exceptional treatment and excellent service and nursing. In return for this they may be looked upon as contributing their diseases for the benefit of medical learning, for no payment is allowed, whether they be rich or poor.

At the same time, a Corporation was set up with increased powers. It included a Board of Trustees as well as the original Board of Directors, the name of the latter having meanwhile been changed to Board of Scientific Directors. The first president of the new Corporation was Frederick T. Gates (adviser to Mr. Rockefeller), and on his death in 1929 he was succeeded by Mr. John D. Rockefeller, jun., who, after twenty-one years service, was followed by his son, Mr. David Rockefeller. As time passed, further generous gifts were made by Mr. John D. Rockefeller and his son (Mr. John D. Rockefeller, jun.).

In 1914, a Department of Animal Pathology was established for the Institute near Princeton University under the directorship of Theobald Smith, who was succeeded in 1930 by Carl TenBroek. In 1931, research in plant pathology was also introduced, so that now the Institute could rightly claim to cover all the main groups of living things. In 1951, the work of the laboratories of animal and plant pathology at Princeton was transferred to the campus in New York.

Simon Flexner retired from the directorship of the Institute in 1935, and he was followed by Herbert S. Gasser, who remained in charge until 1953, when he was succeeded by Thomas M. Rivers. Both Dr. Gasser and Dr. Rivers are now directors emeriti of the Institute, and both still have rooms there.

The year 1953 saw extensive re-organization of the Institute, and Dr. Detlev Wulf Bronk was appointed first president of the Rockefeller Institute of Medical Research. Dr. Bronk is also president of the U.S. National Academy of Sciences, chairman of the National Research Council, and one of America's leading physiologists. He has been professor of physiology in Cornell University and professor of biophysics and director of the Johnson Foundation, University of Pennsylvania. Eventually he became president of the Johns Hopkins University (1948–53) before transferring to the Rockefeller Institute. He is a foreign member of the Royal Society. It has not taken very long to prove that the Institute was indeed fortunate in the election of Dr. Bronk.

In 1953, the Boards of Trustees and of Scientific Directors were merged into a single Board of Trustees which now has fifteen members. They are laymen of distinction in public affairs and men of outstanding scientific achievement, in the ratio of about one to one. These include: Mr. David Rockefeller (chairman), Dr. Detlev Bronk, Prof. R. F. Loeb, Prof. G. H. Whipple, Prof. William J. Robbins, Prof. Philip Bard and Prof. Vincent du Vigneaud.

At present, the staff of the Institute includes twelve members emeriti, all of whom have earned international reputations for their researches in the life sciences; twenty-nine members and professors; seven affiliates; nineteen visiting professors, including such authorities as Lord Adrian of Cambridge (physiology), Prof. T. Dobzhansky of Columbia (zoology), Prof. L. Edelstein of Johns Hopkins (humanistic studies), Prof. Ragnar Granit of Stockholm (neurophysiology), Dr. L. J. Haworth, director of the Brookhaven National Laboratory, Prof. J. G. Kirkwood of Yale (chemistry), [the late] Prof. K. V. Linderstrøm-Lang, Carlsberg Laboratory, Copenhagen (chemistry), Prof. A. M. Monnier of the University of Paris at the Sorbonne (functional physiology), Prof. I. I. Rabi of Columbia (physics), Prof. William J. Robbins,

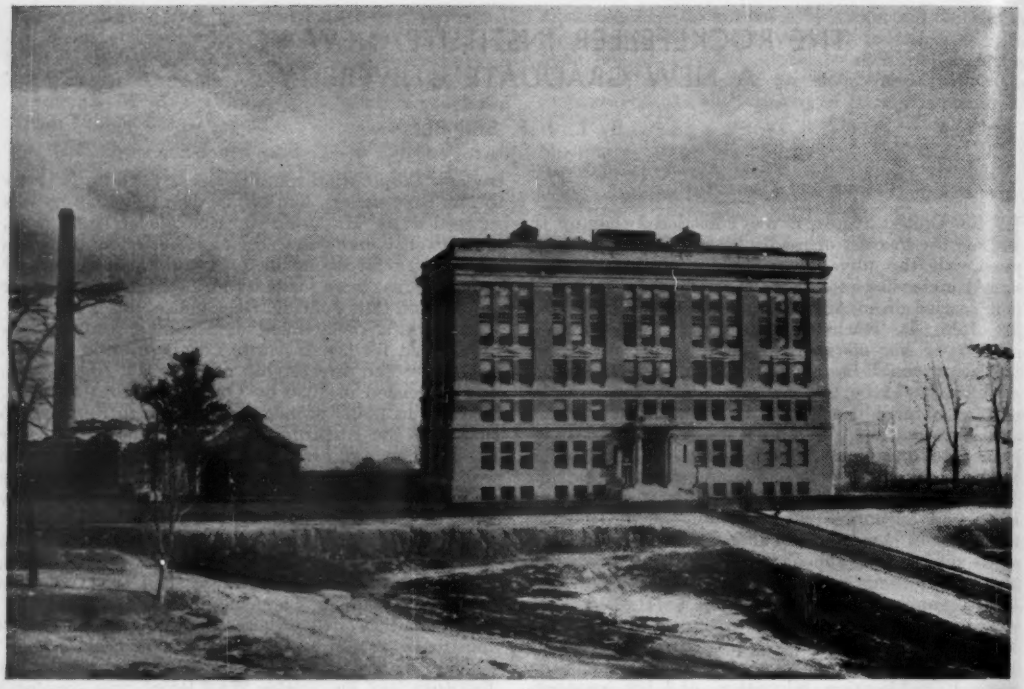

Fig. 1. The Rockefeller Institute for Medical Research as photographed in 1906. Founder's Hall, the only building then in existence

formerly of Columbia (botany), etc.; twenty-four associate professors; thirty-two assistant professors; sixty-two research associates; and forty guest investigators. Prof. Frank Brink is dean of graduate studies, and graduate fellows will this year number 65.

After about fifty years activity, and in order to include graduate education in the natural sciences with special emphasis on the life sciences, the charter of the Institute was amended in 1954 so that it could become part of the University of the State of New York with authority to grant the advanced degrees of doctor of philosophy, doctor of medical sciences, doctor of science (*honoris causa*) and doctor of laws (*honoris causa*). Thus the Institute assumed the status of a graduate university; there are, of course, no undergraduates. It cannot be said, therefore, that the Rockefeller Institute, as a university, is a one-sided affair, for all students have already had those educational and other benefits associated with a full university course before being admitted.

In 1954, the fiftieth anniversary of the opening of the first laboratory, three new buildings were opened, so that then there were Founder's Hall, Welch Hall, Simon Flexner Hall and Theobald Smith Hall, and the Hospital. Since that date, other buildings have been erected and are in use, whereas others are undergoing completion. These are an auditorium, a huge laboratory building, a faculty-visiting scientists social centre, a graduate residential hall, a building for seminars and conferences and a President's House. These buildings and the superb landscaping of the campus (Fig. 3) have been rendered possible by gifts from the estate of Alfred H. Caspary and from Mr. John D. Rockefeller, jun., Mr. David Rockefeller and the United States Public Health Service.

In 1958 the charter of the Institute was amended to change its name from that of Rockefeller Institute for Medical Research to the Rockefeller Institute—a wise decision in view of the comprehensive researches in the life sciences which are now being carried out there.

The current market value of the Institute's endowments is more than 160 million dollars. At present, no more than seventy-five students are to be admitted, at the rate of about twenty each year, to read for the degree of doctor of philosophy or doctor of medical science, so that here we have an aggregate body, a community of scientific scholars, living and working under ideal conditions—a place where they can work, think and dream, away from the sometimes excessive demands made by administration and teaching. The students are few, the faculty (staff) are many. Their creature comforts are catered for in an environment at once functional, beautiful and serene.

The entire organization is of a flexible and personal nature, built around individuals rather than departments, thus allowing the maximum freedom for research and study. The subjects at present being pursued are diverse: they include animal pathology, biochemical genetics, biochemistry, biophysics, biosynthesis, cytology, developmental biology, ecology, general physiology, genetics, history of science, immunology, mathematics, medicine, neurophysiology, parasitology, pathology, physical and organic chemistry, physiology of reproduction and plant pathology.

This academic institution must be unique. It is essential that the graduate students fortunate enough to be accepted be capable of self-directed study. Teaching is mainly done in seminars, tutorial conferences and in faculty research laboratories. The

first three months of each initial academic year are devoted to lectures, seminars and discussions in many fields of natural science. Each week a topic in basic science is discussed by one or more members of the staff. Thus, at the outset of his career the student is enabled to develop a broad foundation of competence in many related fields of science and to grasp the connexions of his special field of research with other aspects of science. Thereafter, the educational programme is designed to suit the needs and interests of each individual student. As soon as the student has formulated a significant problem for investigation, he is encouraged to begin research either independently or under the direction of one or more members of the faculty, knowing full well that extraordinary physical facilities for research are at his disposal.

Moreover, in close proximity to the Institute are opportunities for satisfying interests in the theatre, art, literature, music and other branches of science, and fellowship stipends are of a magnitude to permit students to take some advantage of these (see, for example, *Nature* of May 16, p. 1365). Truly, an international centre of research and culture, for, as the supervisor of one of the Halls remarked: "These students and research workers are also men and women". They look not only inside but also outside the Institute for full development and—the main aim of education—complete living.

First Convocation

The first Convocation of the Rockefeller Institute as a graduate university was held during May 21–22. It opened with a luncheon attended by about three hundred guests, including members of the Rockefeller family, some other prominent Americans and distinguished scientific and academic guests.

Fig. 2. Aerial view of the Rockefeller Institute and its Campus

Top left is the handsome pile of the New York Hospital and Cornell University Medical College, and immediately below this (on the left-hand edge of the photograph) is the corner of Memorial Hospital, behind which is the Sloan–Kettering Institute for Cancer Research. The arrow indicates Theobald Smith Hall of the Institute, behind which is the President's Residence—a beautiful home built in contemporary American style and commanding a magnificent view of East River. Passing to the right are: Flexner Hall; Founder's Hall (see Fig. 1); behind this on the lower slopes facing the River is Welch Hall; the Hospital. On the extreme right are the new laboratories which are nearing completion. Next to this, and facing York Avenue, is the Graduate Students' Residence Hall. Further to the left are Caspary Hall and Abby Aldrich Rockefeller Hall (both included in one building), in front of which is the dome-shaped Caspary Hall Auditorium

In the afternoon, four commemoration addresses (to be published in later issues of *Nature*) were delivered by: Dr. Detlev Bronk, president of the Institute and of the U.S. National Academy of Sciences; Dr. Douglas M. Knight, president of Lawrence College, one of America's leading undergraduate colleges; Lord Adrian, Master of Trinity College and vice-chancellor of the University of Cambridge; and Dr. Henry A. Moe, secretary-general of the John Simon Guggenheim Memorial Foundation and president of the American Philosophical Society.

A reception and dinner were held in the evening, followed by a concert in the auditorium of Caspary Hall. This concert was given by a section of the New York Philharmonic Orchestra conducted by Carlos Surinach. Apart from one item, the pieces played were by contemporary composers—all American except for one Japanese and one Mexican. Introductory remarks by the conductor and the presence of some of the composers rendered this musical occasion one to be happily remembered, for the presentation was exquisite. Even he without an ear for music or he who usually cannot understand or appreciate the modern musical idiom could not help but be impressed by such a full-blooded and enthusiastic performance.

Dancing followed inside and outside the Abby Aldrich Rockefeller Hall under a full moon.

The proceedings on the following day were initiated by the first graduation of this new university—a completely honorary graduation, for the first so-called 'earned' graduation was to be held a few weeks later. The degree of doctor of laws (*honoris causa*) was conferred on the highest academic officers of six of the oldest universities in Europe and the Americas and on Mr. John D. Rockefeller, jun. With the exception of the last-named, the recipients were presented in order of age of the institution they represented.

Dr. G. G. Forni, rector of the University of Bologna, was presented by Prof. R. Dubos, professor of pathology and microbiology in the Institute. Bologna is the oldest of Europe's universities: by tradition it was founded in 1088, though the names of some teachers in Bologna have survived in parchments dating from 1067. By the year 1200, Bologna had some 10,000 students, most of them from foreign lands. Throughout the centuries, this University has been able to count among its alumni such great names as Dante, Petrarch, Copernicus, Malpighi, Margagni, Spallanzani, Galvani and Marconi.

Dr. T. S. R. Boase, president of Magdalen College and vice-chancellor of the University of Oxford, was presented by Prof. G. W. Corner, historian of the Institute. Oxford is the oldest university of the English-speaking world, dating from the reign of Henry II, when, in 1167, foreign-born scholars were driven from Paris. As pointed out by Prof. Corner: "in the year 1649, three hundred and ten years ago, a handful of scholars, vanguard of the new era in experimental science, founded the Philosophical Club, cradle of the Royal Society, exemplar of our National Academy of Sciences". Oxford has been called the 'home of lost causes'; but this is only another way of saying that she is for ever the home of a cause our world must never lose—the cause of liberty to study, think and teach with untrammelled mind in the free city of the spirit.

Lord Adrian, Master of Trinity College and vice-chancellor of the University of Cambridge, was presented by Prof. Peyton Rous, professor emeritus of pathology and microbiology in the Institute. Cambridge also dates back to the twelfth century, and was granted its charter during the reign of Elizabeth I. Oxford and Cambridge "have gone forward side by side throughout almost seven hundred years and have been competitors along most of the way, however impersonal their scholars. Together they emerged from Scholasticism, changed with the Reformation, weathered the Puritan Revolution, the Georgian Era; and yet despite all vicissitudes neither has gained superiority over the other when viewed in the sharp light of the present. Where else does such a state of affairs obtain amongst old universities? Can it be that the love of the English people for contests under fair terms has extended far beyond boat races that almost subconsciously, by giving and taking in a myriad ways, they have maintained the great rival universities in balanced power?"

Dr. J. L. Barandiaran, rector of the University of San Marcos, Lima, Peru, was presented by Prof. R. E. Shope, professor of animal pathology in the Institute. The University of San Marcos was founded in 1551 by royal decree issued by Charles V of Spain. Since that time, it has developed a distinguished record as a centre of intellectual leadership.

Dr. Nabor Carrillo, president of the National University of Mexico, was presented by Prof. Paul A. Weiss, professor of developmental biology in the Institute. This University was also founded in 1551 by Charles V of Spain. It now has a large number of students and teachers working on what must be one of the most beautiful and impressive campuses in the world.

Dr. N. M. Pusey, president of Harvard University, was presented by Prof. A. E. Mirsky, professor of general physiology in the Institute. Harvard, the oldest college in the United States, was founded in 1636 with a gift of £400 from the General Court of the Massachusetts Bay Colony. In the same year, John Harvard, of Emmanuel College, Cambridge, and a Puritan immigrant, bequeathed to the seminary £780 and 260 books; the College was then re-organized, and in 1639 was named Harvard College. Such alumni as Lowell, Longfellow, Agassiz and Emerson have left their stamp on that University; and such presidents as C. W. Eliot, A. L. Lowell and J. B. Conant influenced the entire course of American education.

Owing to his indisposition, the degree of doctor of laws (*honoris causa*) was conferred on Mr. John D. Rockefeller *in absentia*. His son, Mr. David Rockefeller, received the insignia of the degree, after a moving citation by Dr. Detlev Bronk. John D. Rockefeller was born on January 29, 1874, and in 1901 he married Miss Abby Greene Aldrich. They had one daughter and five sons—John D. 3rd, Nelson A. (now governor of New York), Laurance S., Winthrop and David (chairman of the Board of Trustees of the Institute). His life has been devoted to the philanthropic and civic activities of the Rockefeller family, particularly those designed to advance human welfare and those furthering international, inter-faith and inter-racial concepts. He has been active in the organization, administration and policy development of these activities and has made many of them financially possible. He was associated with his father in the creation and development of the Rockefeller Institute for Medical Research in 1901, the General Education Board in 1902, the

Fig. 3. View of the new campus area of the Rockefeller Institute, looking toward New York Hospital. On the left is the Graduate Students' Residence Hall and, on the right, one of the Institute's older laboratory buildings (Flexner Hall)

Rockefeller Foundation in 1913 and the Laura Spelman Rockefeller Memorial in 1918. The last two were merged in 1928. Thus father and son have, as Dr. Bronk said, "been unequalled in their sympathetic understanding, their selfless service, and the scope of their unrestricted, generous gifts in support of scholars and their corporate institutions".

Dedication of New Buildings and Campus

Five new buildings and the North and South Campus were dedicated during the afternoon of May 22. Of necessity in New York, though the buildings are large and spacious, the total area of the campus is comparatively small. This has much to recommend it; for the tendency among some modern universities is to spread themselves over huge areas, thus rendering intercommunication inconvenient if not exhausting.

Caspary Hall contains an auditorium to hold five hundred, numerous seminar and conference rooms, and rooms for the Board of Trustees and senior administrative officers concerned with the educational activities of the Institute. The auditorium is dome-shaped, and its furnishings are of striking beauty. The acoustics are excellent.

Abby Aldrich Rockefeller Hall contains social halls, refectory, library, meeting rooms and private dining rooms for the friendly association of members of the faculty and their families. It also provides living quarters for visiting scientists so that this building may become an international scientific centre for meetings and the exchange of ideas. The air-conditioned residential suites leave nothing to be desired, and the library, refectory and lounges are adorned by some impressive, though controversial, examples of modern American art.

The President's House likewise fosters the development of the Institute as a centre of scientists in a great international city.

The Students' Residence Hall is designed to meet the needs of the most fastidious.

The South Laboratory (Fig. 2) is a huge building which will soon be offering comprehensive facilities for research in many fields.

The campus is charmingly landscaped (Fig. 3).

Of the older buildings, Founder's Hall, Flexner Hall and Theobald Smith Hall are devoted primarily to the housing of 109,000 sq. ft. of laboratories, most of which have been reconstructed and re-equipped within the past ten years. The Hospital, of fifty-six beds, serves as a laboratory for the study of the physiology of man and certain of the diseases which afflict him. Greenhouses provide approximately 10,000 sq. ft. of facilities for botanical research.

Welch Hall contains a library of more than 40,000 volumes, including an unusually fine collection of scientific periodicals. In this building there are also a large faculty dining hall, another for the women of the administrative, secretarial and technical staffs, and social rooms for both groups.

Other buildings comprising the Institute are the Jacques Loeb Laboratories in the Marine Biological Laboratory at Woods Hole, Mass., for those wishing to study marine organisms. Special facilities are also available in the research laboratories of the New York Botanical Garden for those members of the faculty who desire to use the extensive resources there.

Because of increasing dependence of modern scientific research on adequate technical facilities, many supporting services are maintained in order to facilitate and make more effective the work of the Institute. For example, the Animal House contains 40,000 sq. ft. of quarters for animals ranging in size from mice to horses. There are well-equipped shops for the construction of instruments and electronic equipment; whereas shops for machine work and cabinet work make possible the construction and maintenance of all laboratory equipment. Central facilities for electron microscopy, spectroscopic determinations, microanalyses, X-ray studies, photographic work and media preparation are among the many aids maintained for the general use of the various laboratories.

Publications

The Rockefeller Institute Press was established in 1958 and now publishes *The Journal of Experimental Medicine*, *The Journal of General Physiology* and *The Journal of Biophysical and Biochemical Cytology*. Reprints of recent publications are issued periodically as volumes of *Studies*. Events of general interest are published in *The Rockefeller Institute Quarterly*. Monographs and scientific texts are to be published by the Rockefeller Institute Press in association with Oxford University Press.

The celebrations of the first Convocation of the Rockefeller Institute were rounded off by a pleasant garden party in the grounds of the President's House.

In announcing his resignation from the presidency of the Board of Trustees of the Rockefeller Institute in 1950, Mr. John D. Rockefeller, jun., said he had always regarded "this enterprise as the most significant and the most permanent of any that my father established". It is certainly true that the world of scholarship, and especially of science and medicine, owes a great debt of gratitude to the Rockefeller family: particularly through the Rockefeller Institute is the world indebted to John D. Rockefeller, his son John D. Rockefeller, jun., and his son David Rockefeller.

To Dr. Detlev Bronk the world of science and learning will tender their congratulations and express deep admiration for his almost superhuman efforts to further the development and advancement of the Rockefeller Institute and especially for the foundation of this new graduate university.

EVOLUTIONARY SYSTEMS—ANIMAL AND HUMAN*

By Prof. C. H. WADDINGTON, C.B.E., F.R.S.
Institute of Animal Genetics, Edinburgh, 9

THE major advance in the theory of biological evolution since Darwin's day has been the rise of Mendelian genetics. It is scarcely an exaggeration to say that when Darwin wrote biology possessed no theory of inheritance. Since then, the existence of more or less discrete hereditary factors has been discovered, their position in the cell ascertained to be on the chromosomes in the nucleus, and the behaviour of these chromosomes analysed in great detail both in normal and in various more or less unusual conditions.

The whole of this complex of phenomena which are concerned with the manner in which hereditary determinants are passed on from one generation to the next may be referred to as the 'genetic system'. From the point of view of evolutionary theory three main points have emerged concerning it. The first is that the existence of discrete hereditary determinants safeguards variation against being lost if dissimilar animals cross-breed, as Darwin feared it might[1]. Indeed the fact that during the greater part of the life-cycle of most animals and plants the hereditary factors are present in the diploid condition implies that most organisms carry within them much greater potentialities for variation in their offspring than would appear at first sight. The second point, which is one that we owe primarily to the insight of Darlington[2], is that the genetic system itself can be regarded not only as an agent but also as a subject of evolutionary change, which is to say that the genetic system itself evolves. This I shall return to later.

The third point concerns the origin of new variation. It is essential to any theory of evolution that there must be some mechanism by which new variation is brought into being. Darwin was driven to speculate as to what this mechanism might be. In the absence of any understanding of how hereditary qualities are transmitted, he made no pretence that he had found a solution fully satisfactory to himself, although he was somewhat tempted by the theory, usually associated with the name of Lamarck, that characters acquired by organisms in the course of their lifetime might be transmitted to their offspring. Later experimental investigations have shown that in general this is not so.

Our knowledge of the genetic system, which is very extensive and detailed, forces us to the conclusion that the origin of new hereditary variation is to be found only in alterations and rearrangements of the atomic groupings out of which the hereditary factors of the chromosomes are composed. These alterations follow rules of stability which are still entirely unknown to us, but which must be properties of the hereditary molecules themselves and have little or no relation to the precipitating events by which the alterations were stimulated. One of the most firmly based doctrines of modern genetics is that mutation is a random process. I do not wish to challenge this, but I shall suggest later that some care is necessary in interpreting the word random.

* Substance of the Woodhull Lecture delivered at the Royal Institution on February 27.

In present-day biology, evolution is envisaged as resulting from the interaction between, on one hand, the genetic system characterized by random mutation, and on the other, natural selection. The evolutionary pressures exerted by these two factors are exhibited as being quite external to the nature of the organisms involved. The essential evolutionary pressure exerted by the genetic system is that of mutation, and mutation, it is explained, is a random process. Any explanation which might be offered for the nature of the mutational changes would have to be found, it is asserted, in the chemical composition of the genes and not in the nature of the complete biological organism in which these genes are carried. Mutation thus appears as essentially an external force to which the organism passively submits. Again, natural selective pressures are usually thought of as arising simply from the external environment. When the climate changes, a new predator appears, or industrial fumes blacken the tree trunks on which the animal lives, the populations of organisms concerned cannot, it is usually implied, do anything but submit to these pressures and wait until the equally uncontrollable process of mutation throws up a new hereditary variant which enables them to meet the environment's challenge more successfully.

The time seems to have come when we need to take into account two further aspects of the evolutionary mechanism[3]. In the first place, natural selective pressures impinge not on the hereditary factors themselves, but on the organisms as they develop from fertilized eggs to reproductive adults. We need to bring into the picture not only the genetic system by which hereditary information is passed on from one generation to the next, but also the 'epigenetic system' by which the information contained in the fertilized egg is expanded into the functioning structure of the reproducing individual. Each organism during its lifetime will respond in some manner to the environmental stresses to which it is submitted, and in a population there is almost certain to be some genetic variation in the intensity and character of these responses. Natural selection will favour those individuals in which the responses are of most adaptive value.

Two consequences can be expected to follow, and have in fact been demonstrated experimentally. In the first place, natural selection will build up genotypes which set going developmental mechanisms which easily respond to environmental stresses by the production of a well-organized modification which is of adaptive value. It will, as it were, build into the genotype a gun which is not only set on a hair trigger but which is aimed to hit the target when it goes off. In so far as such a developmental response becomes precisely delimited and easily initiated, it becomes the more likely to be produced by unspecified changes in the chemical nature of the hereditary substance. Mutations, which we can think of as random when we are considering the nucleo-proteins of the chromosomes, will have effects on the phenotype of the organisms which are not necessarily random, but which will be modified by the types of instability which have been built into the epigenetic mechanisms by selection for response to environmental stresses.

Since this is an unfamiliar point of view it may be as well to illustrate it by actual example[4]. If eggs of *Drosophila* are submitted to ether vapour shortly after laying, a proportion of them tend to have their development modified so that they produce a very peculiar phenotype, known as bithorax, in which the third segment of the thorax of the animal is transformed from its normal small and obscure structure into a duplicate of the large second thoracic segment. We can treat the response as though it were favoured by selection, and in each generation breed from those individuals in the population which respond to the stress in this way. When this was done with a population taken from a normal wild-type stock, it became apparent that there was some genetic variation of the capacity to respond in this manner. If selection favoured the response, the frequency with which it occurred increased from generation to generation, until after some time it became practically universal when the selected stock was submitted to the ether. After some time a new gene mutation occurred; this was a sex-linked factor which had not been present in the original stock. Its effect is that females homozygous for it lay eggs which tend to develop the bithorax phenotype. This is particularly striking when the factor is present in the selected stock, in which, as it were, the bithorax modification has been set on a hair trigger. If the factor is transferred into an unselected wild-type stock, the tendency to produce bithoraxes is very much reduced. We have here an example in which selection has built up a genotype which exhibits a particular type of developmental instability. A gene mutation has occurred which in the normal *Drosophila* would have only a very slight tendency to produce this phenotype, but which does so with considerable frequency in the selected stock. Thus, if one merely refers to such a gene mutation as 'random', one does not say by any means everything of interest which can be told about it.

The other main evolutionary effect of the epigenetic system depends on the fact that the development of organisms is usually to some extent canalized, in the sense that even though it may become somewhat modified in response to an environmental stress, it also exhibits a tendency to reach its normal end-result in spite of disturbing circumstances If a strain of animals is selected over many generations for its capacity to respond in a particular way to a certain stress, a set of genotypes will be built up in which this response is easily exhibited when the environmental stress is applied, but the developmental resistance to change may be sufficient to ensure that some of it is still retained even if the stress is removed. If that happens, we should have a system which exactly mimics the inheritance of an acquired character, but one which depends not on the direct induction of a hereditary variation, in the manner suggested by Lamarck, but on selection operating on the genetical structure of the population.

Again, there is a practical chapter and verse for such a suggestion in the experiments with the bithorax modification. After rather more than 20 generations of selection for ease of response to ether vapour, the strain had reached a condition in which a high proportion of bithorax individuals appeared even when the ether treatment was not given. This is the process which I have spoken of as the 'genetic assimilation' of an acquired character.

To obtain a complete picture of the evolutionary system, we need to take into account one further set of factors. These may be spoken of as the exploitive system. Animals—the following considerations do not apply so directly to plants—are usually surrounded by a much wider range of environmental conditions than they are willing to inhabit. They live in a highly heterogeneous 'ambiance', from which

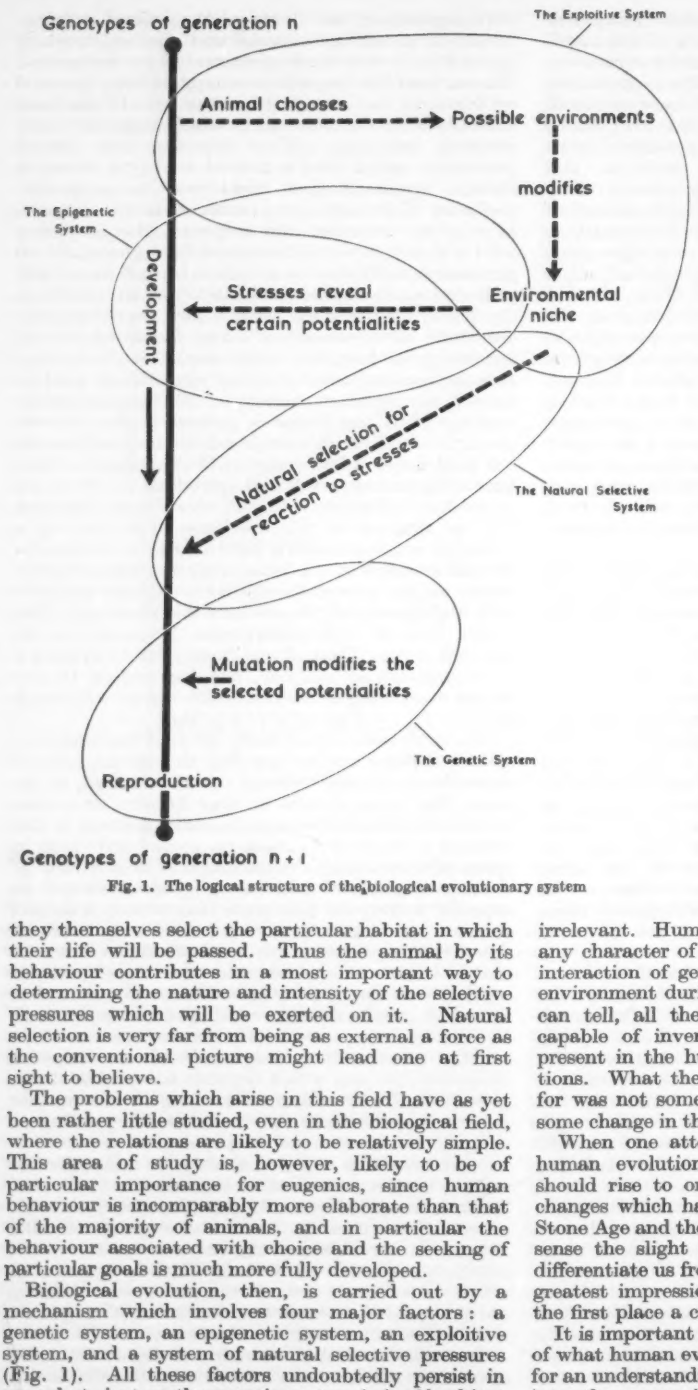

Fig. 1. The logical structure of the biological evolutionary system

they themselves select the particular habitat in which their life will be passed. Thus the animal by its behaviour contributes in a most important way to determining the nature and intensity of the selective pressures which will be exerted on it. Natural selection is very far from being as external a force as the conventional picture might lead one at first sight to believe.

The problems which arise in this field have as yet been rather little studied, even in the biological field, where the relations are likely to be relatively simple. This area of study is, however, likely to be of particular importance for eugenics, since human behaviour is incomparably more elaborate than that of the majority of animals, and in particular the behaviour associated with choice and the seeking of particular goals is much more fully developed.

Biological evolution, then, is carried out by a mechanism which involves four major factors: a genetic system, an epigenetic system, an exploitive system, and a system of natural selective pressures (Fig. 1). All these factors undoubtedly persist in man, but, just as the genetic system is itself subject to evolution, so in broader terms we may say that this whole four-component evolutionary system, of which the genetic system is only a part, itself evolves. The human situation is characterized by an enormously important step in the evolution of the evolutionary mechanism. Indeed, it might be not unreasonable to define humanity by this fact. In man, the processes of teaching by the older members of the population, and learning by the younger ones, have been carried to an incomparably higher pitch than is found in any of the prehuman forms of life, where they play only a relatively minor part, for example, in the determination of bird song, and a few other examples. In man, they have developed not only to highly effective person-to-person learning, as in apprenticeship, but, by the invention of writing and other more recent devices, to an extremely elaborate system by which the whole conceptual understanding of the past is made available to present recruits to human society. We have here what in effect amounts to a new mode of hereditary transmission. It may be referred to as the cultural or 'socio-genetic' system.

Within the past few millennia, the human race has acquired capacities which, in the non-human world, could only have been obtained as the results of evolution. To give a banal example, one may mention the ability to fly. Man has certainly not achieved this capacity by waiting for genes to turn up which have transformed his forelimbs into wings. In fact there is no evidence that changes in the pool of genes available to the human race had any specific effect on the development of the human conquest of the air. This does not mean, of course, that the genes were irrelevant. Human inventiveness and skill, just like any character of any organism, are produced by the interaction of genes with one another and with the environment during development. But so far as one can tell, all the genes necessary to produce men capable of inventing methods of flying had been present in the human population for many generations. What the development of flight was waiting for was not some change in the genetic system, but some change in the cultural system.

When one attempts to consider the problem of human evolution, the type of phenomena which should rise to one's mind are all the most crucial changes which have occurred between, say, the late Stone Age and the present. It is not in any important sense the slight changes in bodily structure which differentiate us from Cromagnon man which make the greatest impression. Human evolution has been in the first place a cultural evolution.

It is important not to overlook this first impression of what human evolution is all about. It is clear that for an understanding of how the human race has come into the possession of those characteristics which we now think most valuable in human life, a theory is needed which is primarily one of cultural evolution.

It seems probable that in the cultural evolutionary system there are parallels for many of the factors operative in the biological system. For example,

parallel to the process of gene mutation there is the arising of new ideas capable of cultural transmission; and there are undoubtedly processes, fulfilling a function similar to that of natural selection, which determine whether newly arisen ideas will in fact be perpetuated in future generations or not. How far is the arising of culturally novel ideas a process which can be characterized as random, even in the restricted sense in which we have seen this term should be used in the biological sphere? What is it that determines the intensity of the natural selective pressure for or against the preservation of new ideas? Again, items capable of cultural transmission such as ideas, techniques or skills do not occur in isolation but are found in co-ordinated groups. Can we find here some parallel to the genetic phenomena of linkage and recombination? The cultural exploitive system, the system of choosing and modifying the environment, is also obviously very highly developed. Again, cultures reacting to external stresses may become particularly liable to modification in certain directions, exhibiting phenomena similar to those we have attributed to the epigenetic system. All these parallels, however, remain as yet very incompletely worked out. It would probably be profitable to pursue them[6].

Although it would seem prudent, when faced by a phenomenon of human evolution, first to consider by what cultural means it has been brought about, it is also very necessary to inquire into the biological or genetical phenomena associated with it. This is, however, exceedingly difficult. Suppose a marked change takes place in the gene pool of the human population; under what circumstances should we expect this to have an important influence on the essential cultural achievements of the group concerned? It is extraordinarily difficult to find any factual evidence, since most examples of marked changes in the gene pool of a population involve the immigration or emigration of individuals who carry with them not only their transmissible genes but also their transmissible culture. For example, one may postulate, as Darlington[5] has done, that the emigrants from Ireland in the middle of the past century carried out of the population many genes for various types of socially effective behaviour, leaving the home population impoverished. But in practice the existence of these genes is only hypothetical, and even if they did exist the emigrants also undoubtedly carried with them culturally transmitted tendencies to vigorous behaviour.

There are a few cases, however—for example, when Jewish populations have immigrated into or out of a country, with whose population they have not engaged in extensive cross-breeding, but on whose life they have exerted a considerable influence—in which one may deduce that this influence has been primarily a cultural rather than a genetic one. Even if we were to make the unsafe assumption that it is their genes which endow the Jewish people or the Chinese with great ability in trade, the effect which a small number of Jews or Chinese may have on the economic existence of the people surrounding them can only be the result of cultural rather than genetic transmission. Again, the rapidity, in terms of numbers of generations, with which many steps of evolution take place (such as, say, the formation of the characteristic United States culture out of an extraordinarily mixed European immigrant population) is scarcely compatible with the operations of the genetic evolutionary mechanism and, it would seem, must in the main be attributed to the actions of the human cultural evolutionary system.

When we can discern cultural differences between two human groups, does the main responsibility of these lie with the system of cultural transmission or with that of genetic transmission? Again, from an *a priori* point of view one must expect differences in both systems to be involved, and it is exceedingly difficult to assess the relative importance of the two contributing factors. It is quite clear that races such as the West Africans, the Maoris and the Chinese, differ genetically from Europeans. Some of these genetic differences are obviously expressed in skin colour; there must surely be others, more difficult to detect. But is there any reason, for example, to suppose that it is the differences in the gene pool between the Chinese and the European populations which have caused the one to develop a social system based on such relatively unindividualistic systems as Confucianism and Buddhism and the other a civilization inspired by such a different system of thought and feeling as Christianity? I see no *a priori* reason to suppose anything of the kind; nor is there sufficient factual evidence to establish such a conclusion.

Again, one can find examples, particularly in quite recent times, in which human populations have undergone important changes in periods of time which seem much too short to allow of noteworthy alterations in their genetic endowment. The change in West Africa from the conditions described a century ago, to the present highly sophisticated and technologically competent modern societies, can only have been accompanied by exceedingly small, if any, changes in the general gene pool. One of the most striking of such changes, and one of the very few that has been carefully studied by a highly trained specialist, is that of the Manus people of the Admiralty Islands in the Pacific, studied by Margaret Mead[7]. This transformation from a palæolithic to at least the beginnings of a modern society has taken place within a lifetime; that is to say, it has involved *no* genetic alteration. It is a most remarkable example of how powerful the cultural evolutionary system may be.

These examples suggest, in my opinion, that in producing the changes of the kind which we consider of major importance in the evolution of mankind, the cultural system of transmission usually contributes incomparably more than the genetical. Even when we can be certain that genetical changes have occurred, as, for example, in the comparison between Africans and Europeans, there are only one or two examples in which these changes can be shown to have any practical importance.

The genetic system of a population, as Lerner[8], in particular, has pointed out, shows a considerable power of resistance to factors such as natural or artificial selection which attempt to alter it. I have argued that the epigenetic system shows similar characteristics of unresponsiveness[3]. One might expect, and I think the evidence suggests, that cultural systems also have some tendency to stability. This is probably correlated to some extent with their size. The fantastically rapid transformation of Manus society occurred in a minute population of only a few thousands; one could scarcely expect the fifty millions of Nigeria or the five hundred millions of China to alter as rapidly. One cannot, therefore, always expect the processes of change mediated by the cultural evolutionary system to occur in a shorter time than those which might be carried out by the

genetic system. It is only in favourable cases that the critical evidence emerges which shows that steps in human evolution can occur at a rate much faster than the genetic mechanisms could bring about. Cultural phenomena which involve stability over long periods need not necessarily, on *a priori* grounds, be attributed to the genetical system; there is no reason to doubt that cultural transmission may operate over many generations. Such questions will only be answerable when we find further methods of gathering empirical data which will enable us to distinguish the contributions of the two information-transmitting systems in each particular case.

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[3] Waddington, C. H., "The Strategy of the Genes" (Allen and Unwin, London, 1957).

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[5] Darlington, C. D., "The Control of Evolution in Man" (Woodhull Lecture). *Proc. Roy. Inst. Vol.* 37; *Nature*, **182** 14 (1958); see also "The Facts of Life" (Allen and Unwin, London, 1953).

[6] Waddington, C. H., "The Human Evolutionary System". Proc. Conference on Darwin and Sociology, Edinburgh, April, 1959 (in the press).

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OBITUARIES

Sir Stanley Angwin, K.C.M.G., K.B.E., D.S.O., M.C.

The death on April 21 at the age of seventy-five of Sir Stanley Angwin brings to a close a remarkable military and engineering career. Educated at Queen Mary College, he received his practical training with Messrs. Yarrow and Co., the shipbuilders. In 1906, at the age of twenty-three, he entered the Engineering Department of the Post Office, a step which took him in the course of fifty years to the highest engineering and administrative positions in the telecommunications field; he became engineer-in-chief of the Post Office in 1939, chairman of Cable and Wireless in 1947 and finally chairman of the Commonwealth Telecommunications Board from 1951 until 1956.

It was provident that Angwin was engineer-in-chief during the Second World War, for the part played by telecommunications was vital and demanded extreme co-operation with the Fighting Services. Angwin had a foot in both the Civil and Services' camps, for he had been associated with the Army Signals since the inauguration of the Territorial Army. His Unit, which he raised as the Lowland Division Telegraph Company, was mobilized in 1914 to form the 52nd Divisional Signal Company and served throughout the First World War in Gallipoli, Egypt, Palestine and France. He was awarded the D.S.O. and M.C., and those who knew his scant personal regard for air raids in 1940 can readily imagine the value and influence of his imperturbable personality as a young commander in the earlier War. His association with Army Signals continued between the Wars, first in command of the 44th Home Counties Divisional Signals and finally as deputy chief signal officer for the Supplementary Reserve.

With this background, Angwin was in an excellent and accepted position to assess and direct the provision of the many long-distance circuits and installations required for the defence of the United Kingdom. The demands for Post Office services were overwhelming, and sometimes competing, particularly for air defence, and later for the U.S. Armed Forces, and the preparations for *D* Day. All this in the face of bomb damage, and incessant repairs, provided a formidable task of direction, while at the same time Angwin fostered the release of technical staff to the Forces for the build-up of the signal staffs and units overseas where their professional knowledge was indispensable.

Between the Wars Angwin's work had been associated with the development of the Post Office and Commonwealth radio services. On his return from the First World War he was appointed to the wireless section of the Engineer-in-Chief's office. There he found, in an era of development, just those opportunities needed to match his many talents. On the technical side he found scope for applying his knowledge and experience in the design and construction of high-power radio stations at Cairo, Leafield and Rugby; and was closely connected with the establishment in 1927 of the first trans-Atlantic telephone service and in the subsequent development of short-wave radio telephone services to all parts of the world.

Angwin's ability in negotiation was equally outstanding. This frequently led to his appointment as chairman of committees, particularly those dealing with controversial matters, at many international telecommunication conferences.

He was chairman of the Radio Research Board during 1947–52. In 1945 he accompanied Lord Reith on a mission to the members of the Commonwealth to discuss changes in Commonwealth communications, leading to the nationalization of Cable and Wireless, Ltd., and to the setting up of the Commonwealth Telecommunications Board.

On retirement from the Post Office, Angwin became in January 1947 the first chairman of Cable and Wireless, Ltd., in its new role, and in April 1951, at the invitation of the partner Governments, followed Lord Reith as chairman of the Commonwealth Telecommunications Board. In 1950, as chief technical adviser to the Board, he had presided over a meeting of technical and traffic experts from all parts of the Commonwealth, which was the first Commonwealth gathering of its kind. He was chairman of the Board for five years, filling this office with his usual distinction and doing much to further Commonwealth co-operation in all spheres of telecommunications. In 1954 he headed a delegation of the Board to Australia and New Zealand. In 1955 he presided over a second successful technical and traffic conference in London, but in 1956, owing to ill-health, he felt obliged to retire.

As engineer-in-chief of the Post Office, Angwin was knighted in 1941 and made K.B.E. in 1945 for his war services. For his services to international telecommunications he was made K.C.M.G. in 1957. He was president of the Institution of Electrical Engineers during 1943–44, Faraday Medallist in 1953 and made an honorary member in 1956. He became a Fellow of Queen Mary College, London, in 1946

and received an honorary degree of doctor of science from the University of London in 1953.

Lady Angwin was an ideal support to him throughout his career. With their family they made their home in Welwyn Garden City, where his charming house and garden must have been a welcome haven in the midst of his exacting duties.

His friends will remember his quiet and seemingly casual manner, his kindly disposition and yet the confidence he could inspire and the force of his leadership.

GORDON RADLEY

Mr. L. E. Harris

MR. LAWRENCE ERNEST HARRIS, a leading authority on fen drainage, died on April 5 at Cambridge, aged sixty-six. The Newcomen Society for the Study of the History of Engineering and Technology has suffered a very severe loss by his unexpected death during his second year as president.

After leaving Mercers School, London, Harris studied at the City and Guilds Engineering College, South Kensington, during 1910–12 and won a Mitchell scholarship. He obtained his practical training with C. Isler and Co., London, artesian well engineers. During the First World War he served in the Royal Fusiliers and the Machine Gun Corps and, as a lieutenant, was invalided out due to war wounds. Having taken a refresher course at King's College, London, Harris joined Sulzer Bros., Ltd., as an estimating engineer in their London office. After three months at their Winterthur Works, he went to India in 1923 as the firm's manager, and for thirteen years was responsible for the sale and installation of many Sulzer steam and diesel engines, pumps and electrical machines in pumping and power stations. Returning to England, he was appointed district manager and engineer for the Sulzer organization in the Fen area in 1937, specializing in fen drainage and pumping machinery. He also installed Sulzer pumps for the Somerset River Board's drainage schemes. He lived in Leeds until 1947, and then in Histon, Cambridge, holding the same appointment until his death.

Harris was intensely interested in the history of his subject, and his authoritative book, "Vermuyden and the Fens", was published in 1953. He wrote the chapter, "Land Drainage and Reclamation" in Vol. 3 of "A History of Technology", published in 1957, and contributed to the Newcomen Society "Sir Cornelis Vermuyden, an Evaluation and an Appreciation" and "Some Factors in the Early Development of the Centrifugal Pump, 1689–1851". In his presidential address, "A Neglected Genius of Seventeenth Century Technology", he paid honour to the inventor, Cornelius Drebbel (1572–1633), of Alkmaar. One of Harris's last interests was to ensure that the beam engine and scoop-wheel installed in 1831 at Stretham, near Ely, by the Butterley Company, should be permanently preserved, as it is the last surviving example of the introduction of steam power for fen drainage. The formation of the Stretham Engine Preservation Trust in 1958 was due to him; he was elected the first chairman of the Trustees and wrote the appeal this year for an endowment fund. His many friends hope the appeal will succeed in its object and that it will be a permanent tribute to his memory. He is survived by his widow, Mrs. Kathleen Harris, and his two sons, Michael and John, both engineers.

A. STOWERS

NEWS and VIEWS

Special Assistant for Science and Technology to the U.S. President: Prof. G. B. Kistiakowsky

PROF. GEORGE B. KISTIAKOWSKY, a distinguished physical chemist, who succeeds Dr. James R. Killian (*Nature*, 181, 384; 1957) as special assistant for science and technology to the President of the United States, spent four years at Princeton University after receiving the Dr.Phil. in Berlin in 1925. He went to Harvard University in 1930, progressing quickly to a professorship in 1937, which he has held ever since. Following early researches on the ultra-violet spectra of polyatomic molecules, and on thermodynamics, after the Second World War he applied shock and detonation waves to the study of chemical problems, determining the heat of dissociation of nitrogen; and employed soft X-rays and mass spectrometers to investigate the phenomena occurring in shock fronts. His most recent studies have been in classical chemical kinetics. Honours that have been accorded to him include the American Medal for Merit (1946), the British Medal for Service in the Cause of Freedom (1948), and the Nichols Medal of the American Chemical Society (1947).

As a civilian scientist during the war years, Prof. Kistiakowsky headed the explosives division of the Manhattan Project, was consultant to the Office of Scientific Research and Development, and adviser to the United States Army on scientific problems of a military character. His wide experience and ability have been recognized by his election to membership in the Philosophical Society and the National Academy of Sciences. In recent years, as a member of the President's Scientific Advisory Committee, his activities and interests have been focused on national scientific policy and on science as a force and a balance in international affairs.

Metallurgy at Manchester: Prof. F. C. Thompson

PROF. FRANK CHARLES THOMPSON, who is to retire from the chair of metallurgy and directorship of the Metallurgical Laboratories of the University of Manchester on September 30, will then have served on the Senate of the University for thirty-eight years. Prof. Thompson graduated in the University of Sheffield in 1911 and was awarded the Mappin Medal; he then served on the staff of the University of Sheffield until 1919, after which he held the Royal Society Sorby Research Fellowship at Sheffield for one year before being called to the chair at Manchester.

At Manchester, Prof. Thompson rapidly established himself as a bold leader of research in his own special subject of the plastic deformation of metals and gave detailed consideration to the atomistics of plastic flow at a time when few physicists realized that a meaningful problem existed in this field of study. He also, and perhaps in the long run this is more important, established himself as a wise academic statesman and a truly sympathetic 'father' to his department. It was a paternity which protected and encouraged the younger members of his staff while

they went their own way in pursuit of their own problems, and gave to the students a basic sense of security spiced with intellectual challenges. The qualities which served the University so well were unstintingly given to the councils of professional bodies, particularly to the Institute of Metals and the Institution of Metallurgists, and he served as president of both these bodies.

Prof. C. R. Tottle

Charles Ronald Tottle, who is to succeed Prof. F. C. Thompson, graduated in 1941 from the honours school of metallurgy at the University of Sheffield with first-class honours. Joining the English Electric Co., Ltd., he was for some years in charge of the metallurgical laboratories of that firm at Rugby. For five years from 1945 he had academic and teaching experience as lecturer in metallurgy at King's College, Newcastle upon Tyne. From 1950 onwards his experience has been in various branches of the atomic energy field, first as scientist in charge of metallurgical developments at Springfield, where, after promotion to senior principal scientific officer, he was appointed assistant director of the Research and Development Branch. In 1951 he was transferred to the new Culcheth Laboratories, of which, in 1955, he was appointed deputy head. In that year he went to Dounreay as head of research and development, and last year became the deputy director and head of the Reactor Division.

Until 1952, Mr. Tottle's publications were mainly concerned with cast iron and residual stress. Two publications dealt with the plastic flow and deformation of cast iron and a third with the general problem of nucleation of cast metals at the mould face. His more recent contributions have naturally been in the atomic energy field and deal with the effect of alloying elements on the creep of metals and the physical and mechanical properties of niobium and its alloys. Much of his work during this period is still 'classified'.

Botany at Aberdeen :
Prof. James Robert Matthews, C.B.E.

Prof. J. R. Matthews, regius professor of botany in the University of Aberdeen, retires at the end of this session. Students and colleagues alike have much cause to thank Prof. Matthews for the unfailing cheerful encouragement and wise council so freely given over a long and distinguished career. Educated at Perth Academy and the University of Edinburgh, Matthews early displayed a deep interest in taxonomy and ecology which has remained throughout his career and has placed him in the forefront of botanists concerned with the problems of the origin and distribution of plants in Britain. Before his appointment as regius professor at Aberdeen, Matthews served as lecturer at Birkbeck College (1913–16) and at the University of Edinburgh (1920–29) and as professor of botany at the University of Reading (1929–34). During the First World War he contributed to our knowledge of the protozoology of dysentery, serving as a temporary protozoologist at the Liverpool School of Tropical Medicine (Western Command). Matthews has given freely of his time to national and local committees as a member of the Nature Conservancy and for a time as chairman of the Scottish Committee, as chairman of the Macaulay Institute for Soil Research (since 1947) and of the Scottish Horticultural Institute (since 1952). He was elected president of Section K for the Brighton meeting of the British Association in 1948.

Prof. Paul Egerton Weatherley

Dr. P. E. Weatherley, who is to succeed Prof. Matthews at Aberdeen, was born in Leicester and received his early education at Wyggeston School; he gained an open scholarship in natural science to Keble College, Oxford, and graduated in the honours school of botany in 1939. Elected to a Keble research scholarship to study fungal metabolism he acted as a part-time demonstrator. Awarded a Colonial Office Scholarship to the Imperial College of Tropical Agriculture (1940), he obtained the A.I.C.T.A. (1942) and was posted to Uganda in the Department of Agriculture. He was in charge of an experimental farm at one of the government research stations and acted as advisory botanist. There he turned his attention to the problems of water relationships of plants and has pursued this as a major research project on his return to Manchester (1947–49) and at Nottingham since 1949. This has culminated in the design and installation of a highly efficient climatological wind tunnel capable of providing a wide range of precisely controlled environments for growing plants (see *Nature*, **183**, 94; 1959). The work has been supported by the Nuffield Foundation and the University of Nottingham. The technique used by Kennedy and Mittler to study the feeding habits of aphids has been applied by Weatherley to study the nature and the movement of solutes in plants. He brings to his new appointment a well knit and vigorously pursued field of experimental botany. Through service on the Board of the Faculty of Science and on University committees, he has acquired at first hand a knowledge of the problems of administration facing universities in this expanding phase of higher education. Apart from botany, Weatherley has a wide range of interests, including painting, hill walking and Scottish country dancing. To his duties as regius professor he brings a wide knowledge of experimental botany, a keen and critical interest in the expanding fields of botany and an appreciation of the difficulties and requirements of students in the modern world of science.

Euratom : British Nuclear Energy Attaché

Mr. D. H. Hill, of the U.K. Atomic Energy Authority, has been appointed resident nuclear energy attaché to Mr. A. H. Tandy, the U.K. Government's representative to the European Atomic Energy Community (Euratom) in Brussels. The Government appointed a permanent representative to Euratom last year. The agreement between the Government and Euratom, which was signed in London on February 4, 1959, provides for close collaboration between the parties for the promotion and development of the peaceful uses of atomic energy within the Euratom community and Great Britain. The nuclear attaché will act as adviser to the British representative and will keep in touch with nuclear developments in all the six member countries of Euratom. Mr. Hill has been a member of the staff of the Authority's Industrial Group since 1956.

Royal Society Artificial Earth Satellites Programme

In accordance with the Prime Minister's announcement on May 12, the Royal Society through its British National Committee on Space Research is engaged in preparing on behalf of the Lord President of the Council a programme of scientific experiments to be carried out in a series of artificial Earth satel-

lites. University departments and other research organizations wishing to propose scientific experiments for incorporation in the programme are invited to communicate with the Assistant Secretary, The Royal Society, Burlington House, London, W.1.

Milk Marketing Board

In 1944 a plan was prepared for an artificial insemination service in all areas of England and Wales except those covered by the pioneer centre that had begun with Cambridge in November 1942. From a 'cow-population' map, territorially convenient blocks of 60,000 cows were ringed off as areas in which to build centres. Centres were to be located on the basis of thirty bulls to each centre (the maximum size because of the risk of foot-and-mouth disease); on the estimate that ultimately 50 per cent of cows in each area would be offered for artificial insemination, and in the belief that 1,000 cows would be inseminated from each bull annually. The last two estimates, thought by many at the time to be far too optimistic, have been confirmed by subsequent history. Already, demand in some areas has resulted in more than ninety cows in every one hundred being inseminated, and the use of bulls is already more than twice the original estimate. In 1959—appropriately the centenary year of the publication of Darwin's "The Origin of Species"—the Milk Marketing Board has announced that 10 million cows have been produced by artificial insemination during the past fourteen years. The way this has been achieved, and the administrative structure of the Milk Marketing Board, is set out in a well-illustrated publication called "Breeding 10,000,000 Cattle" (H.M.S.O.). The Board is rightly proud of its work and tells its story with lively enthusiasm. The fact that a cattle raiser can now breed for a change in the market merely by lifting a telephone is a tribute to the Board and the adaptable British farmer.

New Bell Laboratories

A new laboratory, costing 20 million dollars, will be built by Bell Telephone Laboratories at Holmdel, New Jersey, to meet the expanding need for research and development. Bell Laboratories is the research and development organization for the Bell Telephone System, and employs nearly 11,000 people. The new building will be on a 430-acre site which has been owned by Bell Laboratories since 1929; it has been designed by Eero Saarinen. The first portion of the building is expected to be occupied by late 1961.

Journal of Molecular Biology

This journal is to be published six times a year by the Academic Press, Inc. (London), Ltd. (1, No. 1; April 1959. Subscription £5 per volume), and although published in English, papers in French and German will be accepted. The study of molecular biology attracts scientists from numerous disciplines and with its rapid expansion the problem of correlating results obtained by the specialist has become increasingly difficult. The advent of this new journal with an editorial policy of publishing papers on "the nature, production and replication of biological structure at the molecular level and its relationship to function" should be of great value to workers in this field. With one exception the eight papers and two letters in the first number of the *Journal of Molecular Biology* fall into the framework of molecular biology as defined in the instructions to authors. It is to be hoped that the exception does not represent the thin end of the wedge and that the editors will in future restrict publication to papers dealing with molecular biology and not allow the periodical to develop into another journal of general biochemistry, of which there are already a sufficient number.

The United States National Museum

The annual report of the United States National Museum for 1957–58 (pp. iv+150. Washington, D.C.: Government Printing Office, 1958) records that rapid progress in planning the building for the Museum of History and Technology permitted erection to start in the summer of 1958. The building will be of a rectangular shape having an extended platform to form a terrace at first-floor level. It is significant to note that planning for the interior was undertaken by the architects from draft designs and details submitted by the Museum staff. This is the fifth year of the modernization programme, and the Halls of Health, North American Indian and Eskimos, native peoples of America, and military history have been dealt with. A striking exhibit is that depicting the home life of an average Arapaho Indian family some seventy-five years ago: while the warrior and his wife entertain visitors within a completely furnished tipi, the children play with toys outside and a woman prepares pemmican.

Museum and Art Gallery, Durban

The annual report of the Museum and Art Gallery at Durban for 1957–58 (pp. 19. Durban: Durban Museum and Art Gallery, 1958) states that, in spite of a staff below establishment, much work on display for the public and the re-housing of the scientific research collections has been carried out during the year. The group showing the white-tailed wildebeest and warthog has been re-designed, and work has commenced on the Kruger Park waterhole exhibit. Owing to the vacant keepership in the Art Gallery, work in that branch of the service has been virtually at a standstill, although a few temporary exhibitions have been arranged. In the Old House Museum the court dress and accessories of Sir James L. Hulett have been placed on exhibition, and an interesting tollgate erected in Durban in 1867 has been re-erected in the grounds.

U.S. Atomic Energy Commission: Grants for Training in Radioisotope Technology

Mr. John A. McCone, chairman of the U.S. Atomic Energy Commission, has announced that grants amounting to 251,704 dollars to twelve American colleges and universities will be made for education and training in radioisotope principles and technology. An increase in the number of technical graduates who are able to use radiation as an industrial tool is expected to contribute significantly to the expanding uses of radioisotopes and radiation.

The grants are the first of a series of awards to be made under the Commission's new radioisotope technology training programme. The first series also includes a grant to the University of California, for the purchase of equipment to be used in three industrial radioisotope training courses this summer at Berkeley. Further information can be obtained from the Director, Office of Isotopes Development, U.S. Atomic Energy Commission, Washington 25, D.C.

National Research Council of Canada Support for Universities

Information about the National Research Council of Canada's current programme of university support for science, engineering and medicine is contained in a "Report on University Support, 1958–59" (pp. ii + 143. Ottawa: National Research Council, 1958. 50 cents), which explains the programme, shows how it is conducted, and lists the recipients of scholarships, fellowships and associateships and the direct grants in aid of research. There is also a list of travel grants. Expenditure on scholarships in 1958–59 is estimated at 490,000 dollars, of which 475,000 dollars is for science and engineering and 15,000 dollars for psychology; on fellowships in science and engineering, 360,000 dollars; in medicine, 100,000 dollars; in dentistry, 12,000 dollars; and on medical associateships, 120,000 dollars. Of the grants in aid of research, 2,350,000 dollars is for science and engineering, and 1,250,000 for medical research; 135,000 dollars is allocated for the Prairie Regional Committee, 400,000 dollars for the Atomic Energy Control Board, 136,000 dollars for associate committees, and 366,000 dollars for general research grants. Grants of 43,000 dollars to assist travel by members of university staffs and of 146,580 dollars for special activities at universities bring the total to 5,908,500 dollars, while of a further 749,500 dollars for indirect university support, including administrative costs of the support programme, estimated at 175,000 dollars, 260,000 dollars are for the publication of the *Canadian Journal of Research*.

University College, London: Report for 1957–58

The annual report of University College, University of London, for 1957–58 includes the report of the chairman of the College Committee and that of the provost. This gives a brief account of some of the research work in progress, and a full list of publications by members of the College, arranged by faculty and department, occupies 34 pages of the report. Of the 2,827 undergraduate students and 803 postgraduate students 609 and 338, respectively, were in the faculty of science, 201 and 76 in that of engineering and 190 and two in that of medical science. Of the total, 2,943 were from the United Kingdom, 352 from the the British Commonwealth of Nations overseas and 335 from other parts of the world.

Laboratory Animals Centre

The organization and administration of an animal division for research purposes was considered at a symposium arranged by the Laboratory Animals Centre of the Medical Research Council Laboratories on May 5, 1958. The papers, which have now been published (Laboratory Animals Centre: Collected Papers, Vol. 7; 1958. Pp. 107. Carshalton, 1958. 10*s*.), dealt with the economics and efficiency of an animal division, the relation of an animal division to a research division, the problems of equating supply and demand, the organization of the field laboratories at the University of Sheffield, the scientific research within a laboratory animals division, the staffing of an animals division and animal technician training.

A New Method of Particle-counting on the Electron Microscope

In making estimates of particle counts by electron microscopy it is necessary for the particles from a known volume to be uniformly distributed over the grid in order to get a random sample of the material. In practice, however, this is difficult to achieve, and Backus and Williams (*J. App. Phys.*, **21**, 11; 1950; *J. Amer. Chem. Soc.*, **71**, 4052; 1949), as a compromise, have counted all the particles in small droplets, the size of which was determined by the number of polystyrene latex particles known to be contained in a unit volume of the solution. This technique has recently been improved upon by Nixon and Fisher (*Brit. J. App. Phys.*, **9**, 66; 1958), who used a high-pressure spray gun made of metal, and intended to work with small droplet sizes; it can be used with as little as 0·2 ml. This decreased the size of the droplet, thus making it possible to use much higher magnifications without sacrificing the ability to obtain a picture of each droplet-trace entire on one frame.

Another approach to the problem has now been made by Dr. D. Gordon Sharp, in collaboration with Ivan Sorvall, Inc., Norwalk, Connecticut, in which virus from a known volume is deposited uniformly over a known area of agar jelly by means of centrifugation. The apparatus consists essentially of a centrifuge rotor with eight numbered compartments and a stainless steel cover. It contains eight stainless steel-housed 'Perspex' cell assemblies, and in each cell is placed a 1 cm. square of 2 per cent agar jelly. 1 ml. of the virus is added to each cell and the rotor spun in the centrifuge at 20,000 r.p.m. After spinning the rotor, the cells are opened and the agar squares, which have the virus particles uniformly distributed on their surfaces, removed to dry. A film of collodion is poured over the agar surface, allowed to dry and floated off on water. The floating collodion is then picked up on an electron microscope grid, and the sample is ready for counting. The advantages of this method seem to be that it is simple to use, and, because there are eight cells, eight different samples can be dealt with simultaneously.

Micro-Spot Cathode-Ray Tube

The new Ferranti 5/71 *CM* micro-spot cathode-ray tube has a line-resolving power comparable with that of a well-designed lens and marks an important advance in electron optics. The tube can display all the detail that a lens could project on to the same area and can be used to produce equally clear photographic prints. In systems employing the flying-spot scanning technique, however, resolution in the line direction is limited, for a given scanning speed, by the phosphor decay time, and unless this can be reduced without sacrificing light output it will not be possible to take full advantage of the 5,000 lines in such applications.

Hausaland Erg

From field work and the interpretation of air photographs, A. T. Grove (*Geog. J.*, **24**, 528; 1958) advances the view that the landforms of the plains that stretch along the southern border of the Sahara were shaped by erosion and deposition under climatic conditions that differed greatly from the present, associated with deep weathering, water erosion and the formation of extensive lakes in humid periods followed by phases of much wind action and dune building in the intervening arid periods, matching the alternation of ice and river action in high latitudes. He directs attention, however, to the danger of matching glacial periods with tropical pluvials and to the conflicting views regarding the climatic sequences in the tropics during Pleistocene times in

relation especially to the definition, expansion, or movement of the margins of major arid regions such as the Sahara. Much of the key to this fascinating problem may well lie hidden in the ancient Saharan erg and other sand accumulations of the desert fringes.

Injury-feigning Behaviour of Wood-pigeons

Described as infrequent in the Handbook of British Birds, two apparently related cases of injury-feigning among wood-pigeons (*Columba palumbus*) have been recorded by C. S. Payne (*The Bedfordshire Naturalist*, 12; 1959). Disturbed by a pointer dog in Kempton Park, a wood-pigeon flew straight over the grass at a height of only 1 ft. from the ground, in a manner similar to that of a common sandpiper flying over water. Near the dog was a fully grown and feathered young pigeon which had presumably fallen to the ground before it could fly. Six days later the dog again disturbed an adult wood-pigeon which was "floundering along in the grass as if it had no legs, and progressing a few inches at a time, very awkwardly, by means of its wings". When Payne attempted to pick the bird up it rose buoyantly into the air and flew away. On this occasion no young bird was found. This may have been the same adult which had previously been observed, the distraction behaviour of which had been retained.

Urwick Medal for Management Studies

The Polytechnic Management Association has instituted a new annual award, to be known as the Urwick Medal, for presentation to the best final-year student in the diploma course in management studies at the Regent Street Polytechnic, London. The first award will be made at the end of the session 1958–59. The Association's members are past and present students of management at the Polytechnic.

Royal Society of Tasmania: Officers

The following were recently elected to office in the Royal Society of Tasmania for the year 1959: *President*, The Governor of Tasmania; *Vice-Presidents*, Mr. L. W. Miller, Dr. D. Martin; *Council*, Hon. Mr. Justice Crisp, Mr. R. M. H. Garvie, Dr. E. Guiler, Mr. K. D. Nicolls, Mr. M. S. R. Sharland, Mr. J. W. C. Wyett, Mr. W. F. Ellis, Mr. G. H. Stancombe; *Hon. Treasurer*, Mr. G. E. A. Hale; *Hon. Auditor*, Mr. A. M. Hewer; *Hon. Secretary and Librarian*, Dr. W. Bryden; *Librarian*, Miss E. M. Geddes.

Harwell Reactor School Courses

Eighteen standard or full courses have to date been held at the Harwell Reactor School, and Standard Course No. 19 will start on August 31, and continue until December 18. Altogether, 951 students from 32 different countries have so far attended these courses, which began in September 1954 and are designed to train engineers in the techniques of reactor construction and operation, particularly in connexion with nuclear power stations. Half the places at each course are allocated to overseas students, and a fee of £250 exclusive of accommodation is charged. A special course for senior technical executives will be held during September 21–October 1. This is the ninth course of this kind and the fee is fifty guineas exclusive of accommodation. Application forms and details of both courses can be obtained from The Principal, Reactor School, Atomic Energy Research Establishment, Harwell, Didcot, Berks.

Announcements

Prof. J. A. L. Matheson, Beyer professor of engineering in the University of Manchester, has been appointed as the first vice-chancellor of the new Monash University, Melbourne.

Prof. N. J. Petch, professor of metallurgy in the University of Leeds, has been appointed to the chair of metallurgy at King's College, Newcastle upon Tyne. For a note on the work of Prof. Petch see *Nature*, 178, 347 (1956).

Mr. A. L. Bacharach has been elected president of the Nutrition Society in succession to Dame Harriette Chick. Prof. R. G. Baskett, Mr. F. le Gros Clark, Dr. H. N. Munro and Dr. F. Yates were elected ordinary members of Council. Dr. R. J. L. Allen (Beecham Foods, Ltd., Brentford, Middlesex) is honorary secretary.

The Oliver Bird Prize for 1958 has been awarded to Prof. M. C. Shelesnyak of the Weizmann Institute of Science, Israel, in recognition of his work on the role of histamine in the decidual response of the endometrium and the discovery that ovum-implantation can be suppressed by the presence of histamine antagonists in the uterus before nidation.

A conference on Co-operative Phenomena and Phase Transitions, organized by Prof. G. S. Rushbrooke on behalf of the Physical Society, will be held at Kings College, Newcastle upon Tyne, during July 6–7. There will be four sessions, devoted to: thermodynamics and phenomenology; melting and liquids; quantal fluids; and lattice interactions (Ising and Heisenberg models). Further details may be obtained from Miss E. Miles, Physical Society, 1, Lowther Gardens, Prince Consort Road, London, S.W.7.

The ninth Canadian High Polymer Forum will be held at the Guild Inn, Toronto, Ontario, during October 26–28. The Forum is sponsored by the National Research Council of Canada in co-operation with the Chemical Institute of Canada, and is devoted to all aspects of polymer science. Authors wishing to submit papers for presentation at the Forum should write to the programme chairman, Dr. L. A. McLeod, Research and Development Division, Polymer Corporation Ltd., Sarnia, Ontario, Canada. Titles and abstracts of approximately 300 words will be required by August 1. General information concerning the Forum can be obtained from Dr. M. H. Jones, chairman, Ninth Canadian High Polymer Forum, Ontario Research Foundation, 43 Queen's Park, Toronto 5, Ontario.

The Australian Branch of the Institute of Physics will hold a Conference on Solid State Physics in Melbourne during August 17–22. Some forty physicists from Australia, four from New Zealand, ten from the United States, about five from the United Kingdom, and two from Canada will read papers on original work. The papers offered fall mainly into the fields of lattice and electron theory of metals, low-temperature physics, imperfections and plastic deformation, crystal growth, and surface phenomena. Other fields of solid state physics will also be discussed. Further information can be obtained from the convener, Dr. W. Boas, Division of Tribophysics, Commonwealth Scientific and Industrial Research Organization, University of Melbourne, Carlton, N.3, Victoria, Australia.

FUNCTION OF THE RUMEN

THE 123rd meeting of the Nutrition Society was held at the Royal Veterinary College, London, under the chairmanship of Dr. D. P. Cuthbertson, director of the Rowett Research Institute, on March 7, when a symposium on "Rumen Function" took place. In his opening remarks the chairman described the theme as one of great fundamental and practical interest where the microbiologist, biochemist, physiologist, animal husbandman, veterinarian, and members of the feeding stuffs trade could meet and learn much through the exchange of knowledge and concepts.

Dr. C. C. Balch (National Institute for Research in Dairying, Shinfield) introduced the subject by describing the structure of the ruminant stomach and the movement of its contents. In the course of its development the ruminant stomach has achieved an efficient microbial digestion of plant fibres and also of practically all other plant constituents, and with this goes the syntheses of microbial polysaccharide and protein and also certain vitamins. The structure of the four portions of the ruminant stomach which emerge from the embryonic stomach —the rumen, reticulum, omasum and abomasum— was described. The abomasum with its typical gastric secretory function corresponds to the stomach of non-ruminants; in very young ruminants it is as large as the rumen.

Co-ordinated movements of the omasum, reticulo-omasal orifice, reticulum and rumen can be recorded pneumatically or seen radiographically. These were described by Dr. Balch.

During eating, the food boli are deposited in the anterior rumen, where they rapidly merge. By the end of a meal of hay most of the new food is to be found tightly packed in the dorsal sac as a relatively dry, fibrous layer, whereas the ventral region will contain a layer of more fluid digesta. The reticulo-rumen never empties, and, in fact, in the stall-fed animal rarely contains less than half its capacity. The amounts found in grazing animals are considerably less. The amount of food given, and especially the amount of hay, is a major factor influencing the length of time residues remain in the rumen: there is a slight tendency for concentrates and succulents to pass out of the reticulo-rumen more rapidly than roughages. According to Gordon, the onward passage of particles of hay is greatest during rumination. The transfer of digesta to the omasum is a continuous process.

Dr. B. H. Howard (Rowett Research Institute, Aberdeenshire), in his contribution on the metabolism of carbohydrates by rumen bacteria, first described the various techniques which have been used: (1) *in vivo* studies; (2) 'artificial rumen' preparations; (3) washed cell suspensions; (4) direct microscopic observation including use of fluorescing antibodies; (5) pure culture; and (6) 'enrichment culture' experiments.

Cellulose is the most important of the polysaccharides fermented in the rumen, and xylan occurs in close association with it. Most of the rumen cellulolytic and xylan-decomposing bacteria are very strict anaerobes and some require carbon dioxide and/or rumen liquor. Cellulose yields mainly acetic, lactic and succinic acids in pure culture fermentations, and xylan gives fatty acids, from formic to butyric. Mixed rumen organisms, on the other hand, convert both substances mainly into varying proportions of acetic and propionic acids.

The main rumen starch-fermenter is *Streptococcus bovis*, chains of cells of which can be seen attached to decomposing starch grains. It produces only an α-amylase, and lactic and acetic acids are the chief fermentation products. The same organism will also ferment the oligo- and poly-saccharides containing fructose, found in grass and root crops.

Rumen micro-organisms show a striking capacity to synthesize polysaccharides either internally as iodophilic deposits of 'starch-like' material, or externally as capsules or slime-layers. It is doubtful if the material leaving the rumen to enter the omasum contains any significant amount of digestible carbohydrate of microbial origin.

Lactic and succinic acids added to the rumen are quickly decomposed, yielding mainly propionic acid. Rumen acetic and butyric acids are interconvertible. Formic acid is rapidly decomposed in the rumen to carbon dioxide and hydrogen, which further react to give methane; some five rumen-volumes per day arise from this reaction normally.

In his paper on the utilization of protein by the dairy cow, Dr. M. J. Head (National Institute for Research in Dairying) said that as a result of the action of the ruminal micro-organisms, the value of the nitrogenous moiety of the feed to the dairy cow is not so dependent on its amino-acid structure or its proportion of true protein as is the case with a single-stomached animal. The ammonia produced during the fermentation is either utilized for the growth of organisms which are later digested to give nutrients of high biological value, or is absorbed through the rumen wall into the blood-stream and largely wasted. This loss has not been sufficiently considered in evaluating protein feeds for ruminants.

The classical assessment of the biological value of a protein is complicated in the case of a ruminant by the fermentation in the rumen, as has been shown by the work of Chalmers and Synge. The greater the loss of ammonia from a protein, the greater the significance to the animal of the amino-acid structure of the feed protein residues leaving the rumen unattacked.

A comparison of the various oil-cake residues normally used as protein sources for dairy cows on the basis of daily *in vivo* rumen ammonia concentration curves did indicate differences between the cakes. A similar classification of these protein sources could be made on the basis of the proportion of their total nitrogen soluble in 1 *M* sodium chloride solution.

While theoretically the most desirable type of protein for a ruminant is one of low solubility in the rumen and yet of high biological value for a single-stomached animal, two further questions need consideration with reference to practical feeding. First, is the dairy cow normally rationed sufficiently stringently for any of these theoretical improvements to be seen to advantage; and secondly, may it not be more economic to feed a theoretically wasteful protein source if it still supplies cheaper units of biological value?

In discussing lipids in relation to rumen function, Dr. G. A. Garton (Rowett Research Institute) pre-

faced his paper by a consideration of the nature of dietary lipids. The lipids present in feeding stuffs such as grass, hay, silages and concentrates were considered with special reference to the C_{18} poly-unsaturated fatty acids (linoleic and linolenic) which form a large part of the total fatty acid content.

Events in the rumen were considered first with reference to the hydrogenation of unsaturated fatty acids, which occurs to a considerable extent under the influence of the rumen bacteria. This results in the production, *inter alia*, of stearic acid which, following intestinal absorption and deposition, may well account for the characteristic 'hardness' of ruminant depot fats.

Recent findings on the hydrolysis of triglyceride fats in the rumen by a bacterial lipase were then outlined. These demonstrate that most of the fatty acids entering the rumen as glycerides reach the abomasum and small intestine in the free state—a situation markedly different from that in monogastric animals in which partial lipolysis takes place in the small intestine. The fermentation of the liberated glycerol (to yield propionic acid) was briefly discussed.

The ruminant does not appear likely to derive any of the so-called 'essential' fatty acids from the lipids of its rumen bacteria. Thus it appears that, if the ruminant requires 'essential' fatty acids, they must be of dietary origin and must also escape hydrogenation in the rumen.

In his contribution on milk composition in relation to rumen metabolism, Dr. J. A. F. Rook (National Institute for Research in Dairying) indicated that the observations of Powell in 1938–41 provided convincing evidence that the fine grinding of the entire roughage in a ration or, alternatively, a considerable reduction in roughage intake and an increase in the concentrate feed, reduced the percentage and yield of fat without affecting total milk production. This specific effect on fat secretion has been fully confirmed by Balch, Balch, Bartlett, Cox and Rowland at Shinfield. Milk of unusually low fat content was observed by McClymont to result from cows grazing on young herbage or fed on kale, concentrates and limited amounts of hay or straw, that is, diet short of roughage. The operative factor is the physical condition of fibrousness rather than the presence of fibre.

The depression in the milk fat secretion of cows on low roughage diets has invariably been associated with a change in fat composition. Milk fat in the cow and other ruminants is distinguished from the milk fat of other species, and from animal depot fats in general, by a relatively high content (up to 20 per cent on a molar basis) of short-chain (C_4–C_{10}) acids, and when a reduction in fat secretion occurs the proportion of these acids is correspondingly reduced. Some reduction in the weights of higher saturated (largely palmitic and stearic) and also unsaturated (largely oleic) acids secreted is also observed.

Additions of sodium acetate (500–1,500 gm./day) or acetic acid have produced an almost complete recovery in the fat content of milk, but individual animals have failed occasionally to show a response to sodium butyrate. It is held to be the change in the production of acetic and propionic acids that has generally been responsible for the depression in milk fat synthesis.

The role of the protozoa was outlined by Dr. Margaret Eadie (Rowett Research Institute) in her paper on some aspects of rumen ciliate protozoa. According to Dr. Eadie, the basic nature of the association of rumen ciliates with rumen function is a complex one still not clearly understood, but their study is valuable because of the one justified generalization: that certain species of ciliates have been found in the rumen of all normal adult ruminants so far examined.

In general, diet appears to have the greatest effect upon the ciliate population, and variations are so large that no acceptable average figure can be given nor can the ciliate protein contribution be accurately assessed. This may be as high as 20 per cent of the total host requirement, and qualitative studies have indicated that the ciliate protein is of higher nutritional value than the rumen bacterial protein.

Comparisons of faunated and non-faunated animals have so far shown no serious effects on those free from ciliates, but it has yet to be ascertained whether advantage from a ciliate population would appear under conditions of stress. In recent work in collaboration with Dr. Preston at the Rowett Institute, experiments are in progress to determine, by variation of the roughage content of the weaning ration, how soon ciliates may be permanently established in early-weaned calves and whether this is advantageous.

Numerically the oligotrich organisms, particularly *Entodinium* spp., make up the largest proportion of most populations. So far, the holotrichous organisms, which are fewer in number, have been most closely studied and the information of the biochemical and nutritional properties of these organisms suggests that equally interesting information should accrue from similar studies of the oligotrich ciliates.

In his paper on the treatment and prevention of bloat with anti-foaming agents, Mr. C. S. W. Reid (Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand, at the Physiological Laboratory, Cambridge) pointed out that if stable foams are generated during fermentation, the elimination of gas from the rumen is impeded and bloat (tympanites) may result. His paper reviewed work carried out in New Zealand on the use of anti-foaming agents in the treatment and prevention of acute bloat in grazing dairy cattle. Administration of foam-breaking substances—fats and oils, certain detergents and other surface-active materials but not silicones—was the only consistently useful treatment found. Materials at present recommended for field use are peanut oil and emulsified ruminant tallow in doses of 120–150 gm. fat. Anti-foaming agents were also effective when given prophylactically. Pasture spraying proved to be a reliable control measure and has been used on New Zealand farms over four seasons. It has also been notably successful in preventing bloat on irrigated pastures in Australia.

Detectable anti-foaming activity is present in the rumen contents and can be shown to be affected by the activity of the micro-organisms. However, apart from indicating the probable complexity of the situation, observations so far have not revealed obvious correlations between lipid intake, anti-foaming activity and bloating.

Dr. A. T. Phillipson (Rowett Research Institute) in his paper on the rumen in relation to the animal considered first the extent of digestion in the reticulum and rumen. He pointed out that several estimates have been made of the extent of digestion in the reticulum and rumen which are based on the lignin ratio and which suggest that the quantity of food constituents disappearing ranges from 43 to 83 per

cent in cattle and that a similar but rather narrower range has been found in sheep. Most of the material disappearing is carbohydrate. It is more difficult to assess the extent of disappearance of nitrogen-containing materials as the secretions augment the nitrogen of the food, but it appears that considerable losses occur from the rumen in both cows and sheep and presumably these represent the absorption of ammonia. However, losses in the intestine are more extensive when compared with the apparent digestibility of nitrogen for the whole alimentary tract.

Estimates of fatty acid production in the rumen show that they are quantitatively important. The recent work of Armstrong and Blaxter at the Hannah Dairy Research Institute indicates that at levels of feeding up to maintenance the value of the mixture of short-chain fatty acids to the animal as a source of energy is fairly constant irrespective of the quantities of each individual acid absorbed. For production purposes, rations that favour the formation of propionic and butyric acids should be more valuable than those that favour the production of acetic acid. The proportions of propionic and butyric acids increase as the concentrate part of the ration increases in cattle. In particular, starch-rich food increases the proportion of propionic acid. As yet, very little is known of the peptic and intestinal phases of digestion.

In the second part of his paper, Dr. Phillipson dealt briefly with the absorptive capacity of the intestinal tract. One of the conditions of terrestrial life is the necessity of conserving water and sodium, and the ruminant is unusually good at carrying out these two tasks.

The quantities of water and sodium secreted in the saliva alone are far greater than the quantities excreted in the faeces. The contribution made by other digestive juices still needs investigation and is likely to be considerable. Both sodium and water are known to be absorbed from the rumen and omasum, while there is no doubt that absorption also occurs along the remainder of the tract, particularly in the large intestine. It is of particular interest that in conditions of sodium depletion the sodium content of the saliva is lowered and its place is taken by potassium; there is also a diminution of the volumes secreted. A study of the rates of turnover of body constituents such as water, sodium and other elements and nitrogen in various circumstances may help in the end to understand why occasionally things go wrong.

The final paper of the symposium was by Mr. J. K. D. Dow (Unilever, Ltd.) on the application of recent scientific information through the feeding-stuffs industry.

Studies on the absorption of fatty acids in the rumen, and the connexion with hormone metabolism, indicate dietary means of regulating milk production and quality and of curing conditions such as ketosis. Chemical tests enable processed protein materials to be classified according to the needs of the animals for which they are best suited. The feeding-stuffs industry has already made use of research into the vitamin and mineral requirements of ruminants. Further research on the effect of these on roughage utilization is awaited.

Recent work on antibiotic supplements for calves has indicated results which are not applicable through the industry in the present stage of legislation. This case demonstrates the wide view which is demanded of the research worker to-day, particularly when some manufacturers may depend entirely on published scientific evidence in formulating new feeding stuffs. However, it is doubtful whether fundamental research workers have adequate facilities for testing their results under practical conditions. This problem offers a profitable field for consideration by the feeding-stuffs industry, because co-operation between the research worker and the manufacturer is essential to the efficient functioning of the industry and, ultimately, will ensure that the farmer reaps the fullest benefit.

D. P. Cuthbertson

NUCLEAR FUEL CYCLES

THE Institute of Physics, in collaboration with the British Nuclear Energy Conference, held a symposium on "Nuclear Fuel Cycles" at the Institution of Civil Engineers, Westminster, on January 22 and 23. The symposium was opened by the president of the Institute of Physics, Sir George Thomson, who welcomed some 250 members and visitors, including delegates from eight European countries. He stressed the importance of fuel cycles in relation to the large nuclear power stations now under construction in the United Kingdom. These would prove competitive with coal-fired stations only if their uranium fuel were employed in the most efficient manner.

The symposium was divided into three sessions, the first of which considered the basic physics of long-term reactivity changes and nuclear fuel cycles, the second was concerned with the optimization of fuel cycles, and the third session discussed relevant operational problems. The chairman of the first session was Dr. J. V. Dunworth (Atomic Energy Research Establishment, Harwell). The first paper presented was that by Dr. H. Rose and Mr. J. J. Syrett (Atomic Energy Research Establishment, Harwell) on "Long-term Reactivity Changes", which described the basic physical processes occurring as natural or slightly enriched uranium is irradiated in large graphite-moderated thermal reactors. The importance of these changes was emphasized since, together with considerations of metallurgical damage, they would determine the irradiation-level at which the fuel must be replaced. The overall reactivity-changes with irradiation are determined mainly by the small net difference between the large changes arising from the burn-up of uranium-235 and the formation of plutonium-239, making the problem unusually sensitive to all the relevant nuclear data. Furthermore, the plutonium isotopes formed in the irradiated uranium have large resonances in their fission and capture cross-sections at low neutron energies. The prediction of their rates of reaction with neutrons therefore demands a detailed knowledge of neutron spectrum conditions to an extent unnecessary in assessing the initial reactivity of the reactor. For these reasons, present predictions rely considerably upon integral measurements which are made on irradiated fuel

elements. Much of this work has been carried out using the *Gleep* pile oscillator at Harwell, where data have been accumulated from uranium which has been highly irradiated in the Windscale, NRX and Calder Hall reactors. Typical predictions were displayed of the long-term reactivity changes to be expected for an individual fuel element in the Calder Hall type reactor, and the dependence of the reactivity curve upon several important design parameters was also illustrated.

In the ensuing discussion, Mr. P. W. Mummery (Atomic Energy Research Establishment, Harwell) explained that the long-term reactivity changes occurring at Calder Hall have been observed directly. Their interpretation was difficult since they applied to a complete reactor, and it was necessary to allow for the effects of different irradiation-levels throughout the reactor and the variation with irradiation of the control-rod effectiveness. Even so, there were some grounds for satisfaction that these measurements were in reasonable agreement with the *Gleep* experiments. Finally, he emphasized the very high accuracy on the basic nuclear data which is demanded for a purely theoretical approach to these problems.

For a given flux distribution and fuel-cycle scheme, the long-term reactivity changes of an entire power reactor may be found by suitably weighting the contribution from each fuel element. This was discussed in the second paper, by Mr. S. E. Lewis (Nuclear Power Plant Co. Ltd.). He considered the basic theory of 'once-through' fuel cycling, in which the fuel supplied to the reactor has a constant composition and does not contain any materials produced during the irradiation of previous charges. Mr. Lewis showed how an equilibrium state is reached in a small region where the flux is uniform and maintained constant, and demonstrated the factors determining the reactivity of the region and the whole reactor during the approach to the equilibrium state. The equilibrium state is determined when fuel is added and removed continuously in such a way that in any region of the reactor all irradiation-levels are represented between zero and the maximum allowable. Certain basic fuel-cycle schemes were outlined, some of which necessitated fuel movement within the reactors. It was concluded that the delay of commencement of continuous fuel replacement could be economically advantageous, and warranted closer study.

It is necessary to investigate the local behaviour of reactor flux and heat output in the small region considered in the previous paper, as the fuel elements will have different irradiation histories. This problem was discussed in a paper on perturbations due to fuel cycles which was presented by Dr. J. G. Balfour, Dr. D. L. Booth and Mr. R. S. Bulloch (Atomic Power Projects). One-group perturbation theory was applied to the calculation of axial flux changes in a single channel caused by the non-uniform irradiation and temperature, and by axial fuel shuffling within the channel. An improved method was then developed in which the non-linear one-group diffusion and heat transfer equations were solved numerically, this being applicable even when the perturbations were large. Axial distributions of flux and of coolant temperature were illustrated both before and after neutron irradiation of the fuel. A final section of this paper described the use of the Feinberg method for estimating local channel-to-channel variations of power and flux during a continuous fuelling scheme. The work suggested that although the fission cross-section of a fuel element varied considerably over its life due to build-up of plutonium, the heat output maintained a more constant level due to the increased depression of the neutron flux.

In the discussion, Mr. Syrett pointed out that the choice of fuel cycle had an important bearing upon reactor economics. In the approach to equilibrium which lasted several years, large sums of money could be saved by a judicious choice which would reduce fuel requirements and investment charges. He explained the potential advantages of radial shuffling for giving improvements during the approach period and in the equilibrium state.

In summing up the session, the chairman emphasized the importance of reaching a practical and economic fuel-cycling procedure for the Central Electricity Generating Board reactors, one which would have to take into account many different technologies. It was imperative that during the first few years of operation of these reactors, intensive operational research should be performed to improve our knowledge of fuel-cycling problems.

The second session (chairman, Mr. L. Rotherham of the Central Electricity Generating Board) contained two papers concerned with the optimization of fuel cycles. The first, by Mr. P. R. J. French and Mr. C. S. Lowthian (A.E.I.–John Thompson Nuclear Energy Co. Ltd.), considered a once-through equilibrium fuel cycle. The influence of the fuel cycle on the optimization of the reactor design through the lattice parameters was studied, and a relationship developed between burn-up of fuel and the initial neutron spectrum conditions. The effect of enrichment upon fuel life was also discussed, together with the allocation of excess reactivity to increase reactor output or fuel life.

The second paper was presented by Dr. P. J. Grant, Mr. W. Oswald and Mr. P. D. D. Russell (General Electric Co. Ltd.) This examined economic factors affecting the choice of fuel cycle for nuclear power stations, and underlined certain aspects often overlooked. Optimization of the fuel cycle is only a part of the overall optimization of cost per kilowatt hour, which depends partly upon local conditions. Neutron economy was important, but might sometimes conflict with monetary economy. As an example, flux flattening with steel absorbers and attendant loss of neutrons at the reactor centre could yield longer fuel life and reduce power costs. The economy of enrichment, plutonium production and fabrication costs was considered, as were the present prices of nuclear fuels. The advantages of axial fuel shuffling in the Calder Hall type reactor, and the necessity for continuous charge and discharge schemes, were also discussed. It did not appear generally economic to reduce deliberately the irradiation-level below the metallurgical limit.

Mr. G. E. Darwin (Babcock and Wilcox Ltd.) commented in discussion on the importance of this limit. The uranium and canning materials would not stand up well to fuel shuffling and the necessity to operate under differing physical conditions. This would result in reduced metallurgical limits depending upon the shuffling schemes, and it was inadvisable to assume a constant limit in fuel-cycle considerations. Mr. Syrett pointed out that delaying the onset of the equilibrium charge – discharge scheme could reduce fuel requirements, and since potential savings of the order of £1 million per reactor were possible this provided an incentive for such schemes. He asked Mr. French and Mr. Lowthian whether large

computer programmes were preferable to their analytical approach to fuel cycle considerations. Mr. Lowthian replied that it might be difficult to analyse all the information provided by the machines, but agreed that machine effort was extremely useful. In response to a question by Dr. G. Davis (Israel), Mr. Mummery stated that one could not dismiss fuel cycling and on-load charge – discharge machines as luxuries, since these provided a potential reduction of the order of a factor of two in fuel costs as well as making it possible to discharge burst cartridges without losing output.

A paper on the operational problem associated with fuel cycling was presented by Mr. J. C. C. Stewart and Dr. N. L. Franklin (Risley) in the third session, for which Prof. J. Diamond (University of Manchester) was in the chair. The authors pointed out that unlike some other industries, conservative operating procedures based upon precisely specified codes of practice cannot be expected because of the rapidity of development in the reactor field. It is essential in their opinion to choose an irradiation life-time which represents a compromise between metallurgical failure-rate and reduction in fuel costs. Sums of the order of £1 million per charge might depend upon this choice. They believed that the reactor operator and fuel manufacturer must carry out a sequential experiment to study the development of fuel cycles and ultimate life of fuel elements, using the actual power reactor itself as the test vehicle. This would also allow for the introduction and testing of subsequent types of fuel element as they are developed. The authors pointed out that this would have considerable influence upon the fuel fabrication facilities required.

Dr. C. P. Haigh (Central Electricity Generating Board) remarked that as a reactor operator he had special problems to consider such as on-load refuelling. The high capital costs of reactors had to be offset by low fuel costs and a high load factor. Shut-down of one reactor for one day would cost £8,000, while a prolonged shut-down involving a complete fuel discharge would cost around £2 million. Thus the reactor operator must be somewhat conservative initially in relation to fuel cycling. Experience would be accumulated gradually on reliability, before large-scale use would be made of complex fuelling schemes.

The closing remarks at the end of the symposium were made by Mr. P. W. Mummery, who explained that the main aim of the symposium had been to indicate the implications of the basic physics problems involved in nuclear fuel cycles on the design and operation of reactors. Compromises are necessary between conflicting requirements from many fields such as physics, metallurgy, engineering, heat transfer, operational simplicity, etc. The potential advantages of various types of fuel cycle had been indicated, on the assumption that various kinds of fuel movement within the reactor were possible and that the irradiation life was limited only by reactivity. Although such cycles could not be used initially, until more experience and more information had been gained, the design of reactors should be flexible to allow for developments such as these. It would be many years yet before the best fuel cycle was established for the Calder Hall type reactor. He looked forward to a time, three or four years hence, when actual operating experience of fuel cycling on different systems might be reported at a similar symposium.

The papers presented at the symposium and a summary of the important discussion will be published in the July issue of the *Journal of the British Nuclear Energy Conference*.

H. Rose

THE ROYAL AERONAUTICAL SOCIETY

AWARDS FOR 1958

THE following awards have recently been made by the Royal Aeronautical Society: *Honorary Fellowship* has been conferred on Prof. J. Ackeret (professor of aerodynamics at the Federal Technical College, Zurich), Sir William S. Farren (technical director, A. V. Roe and Co., Ltd.), S. B. Gates (chief scientific officer and consultant to the director of the Royal Aircraft Establishment); *Honorary Companionship* has been conferred on E. C. Bowyer (director and chief executive of the Society of British Aircraft Constructors), Sir William Hildred (director-general of the International Air Transport Association).

Silver Medal of the Society has been awarded to Dr. E. A. Watson, director of Joseph Lucas (Gas Turbine Equipment), Ltd., for his achievements in the development of turbine engine combustion and fuel systems; *Society's Bronze Medal* to D. G. King-Hele, of the Royal Aircraft Establishment, Farnborough, for his contributions to Earth satellite orbital theory; *British Gold Medal for Aeronautics* to R. S. Stafford, technical director, Handley Page, Ltd., for his outstanding practical contributions to aircraft design; *British Silver Medal for Aeronautics* to D. J. Farrar, chief designer, Guided Weapons, Bristol Aircraft, Ltd., and Dr. N. H. Searby, manager, Guided Weapon Research and Development, Ferranti, Ltd., for their practical contributions to the development of guided weapons; *Wakefield Gold Medal* to K. A. Wood, superintendent in charge at the Atomic Weapons Research Establishment, Orfordness, for his practical achievement in the development and application of electronic aids to all-weather approach and landing; *R. P. Alston Medal* to A. W. Bedford, chief test pilot, Hawker Aircraft, Ltd., for his contributions to the technique of flight testing high-speed aircraft; *N. E. Rowe Medal*, for the best lecture given before any branch by a member of a branch, to J. Dunham, Rolls-Royce, Ltd., for his paper on "Damage to Axial Compressors" given before the Derby branch of the Society; *Simms Gold Medal* to Mr. R. P. Probert, deputy director, Engine Test Facilities, National Gas Turbine Establishment, for his paper on "Ram-Jets"; *George Taylor (of Australia) Gold Medal* to Mr. H. L. Cox, of the National Physical Laboratory, for his paper on "The Application of the Theory of Stability in Structural Design"; *Edward Busk Memorial Prize* to Mr. T. R. F. Nonweiler, of The Queen's University, Belfast, for his paper on "The Man-powered Aircraft

—a Design Study"; *Orville Wright Prize* to D. A. Spence, of the Royal Aircraft Establishment, Farnborough, for his papers, "The Lift on a Thin Aerofoil with a Jet-augmented Flap" and "Some Simple Results for Two-dimensional Jet-flap Aerofoils"; *Herbert Ackroyd Stuart Memorial Prize* to Mr. A. A. Lombard, director of engineering, Aero-Engine Division, Rolls-Royce, Ltd., for his paper on "Thinking about Aircraft Engines"; *J. E. Hodgson Prize* to Mr. J. E. Allen, head of Aerodynamics, Project and Assessment Department, Weapons Research Division, A. V. Roe and Co., Ltd., for his paper, "From Aviation to Astronautics"; *Branch Prize* to Mr. B. D. Blackwell, engineering manager, Bristol Aero-Engines, Ltd., for his paper on "Some Investigations in the Field of Blade Engineering"; *Navigation Prize* to Mr. C. M. Cade, of the Research Department of Kelvin and Hughes, for his paper on "Radio-Astronomy and Navigation"; *Usborne Memorial Prize* to Dr. J. S. Przemieniecki, of Bristol Aircraft, Ltd., for his paper on "Matrix Analysis of Shell Structures with Flexible Frames"; *Pilcher Memorial Prize* to Mr. A. W. Kitchenside, of Vickers-Armstrongs (Aircraft), Ltd., for his paper on "The Effects of Kinetic Heating on Aircraft Structures".

NEW NATURE RESERVES IN BRITAIN

THE Nature Conservancy has recently announced the establishment of four new nature reserves and one forest nature reserve.

The Rhinogs Nature Reserve. The Harlech dome and its borders display the thickest succession of Cambrian rocks to be seen in Britain. It forms a region of desolate and almost uninhabited moorland with high, rugged block-like mountains where the Rhinog Grits break the surface. The two principal peaks are Rhinog Fawr (2,362 ft.) and Rhinog Fach (2,330 ft.). The new reserve covers two blocks of these uplands, lying on either side of Bwlch Drws Ardudwy about five miles east of Harlech, which together total about 991 acres; it is hoped that it will ultimately be extended to include other areas of outstanding scientific interest in the area. The first of these blocks comprises the northern slopes of Rhinog Fawr from the peak down to about 1,066 ft., and the second includes the peak and north-eastern slopes of Rhinog Fach down to the 1,000-ft. contour. The terrain is rugged and wild in the extreme, with a considerable amount of exposed rock in the form of steep crags, broken slopes and block screes. A flock of wild goats, thought to be of domestic origin, frequents the Bwlch Drws Ardudwy area, and pine martens have been seen there in recent years.

Compared with other parts of Snowdonia, the Rhinogs are relatively lightly grazed by sheep, and this may be related to the abundance and luxuriance of heather which extends on these hills from about 600 ft. to the summits. The rocky, well-drained slopes up to the 1,500-ft. contour are covered with dense heather, mixed here and there with bilberry, both reaching 5 ft. in height in the more sheltered situations. The less rocky well-drained areas bear patches of acidic hill grassland, mainly of the *Agrostis–Festuca* type, with some bracken. Wherever hollows and stream flushes occur small bog patches are found, and the wettest ground is occupied by blanket bog with peat up to 4 ft. deep. The small summit plateaux and the steep craggy uppermost slopes of the two Rhinogs bear much block scree, and here the heath forms dense prostrate mats with unidirectional growth away from the prevailing south-west winds.

Access to the reserve is unrestricted, but those wishing to collect specimens or to carry out research should apply for permits to the Regional Officer for North Wales, The Nature Conservancy, Y. Fron, The Crescent, Upper Bangor, Bangor, Caernarvon.

Coed Dolgarrog Nature Reserve, which lies about seven miles south of Conway, is an excellent example of an oak wood on the drier north-eastern side of Snowdonia; many of the trees are intermediate in kind between the sessile and pedunculate oak. Viewed from the floor of the Conway Valley, these woods are probably the most impressive in North Wales; they clothe a precipitous slope which falls from 1,000 to 100 ft. It is unlikely, because of the topographical features of the area, that the woods have been subjected to any form of intensive land use. The reserve, most of which lies within the Snowdonia National Park, covers 170 acres and has been established under a lease from the Central Electricity Generating Board.

Coed Dolgarrog is of particular interest ecologically because of the variety of underlying rocks and the clarity with which their effects on the vegetation are displayed. The central part of the wood lies on lime-rich volcanic ash and has a luxuriant ground flora including much dog's mercury, slender false-brome and sanicle. At higher altitudes elsewhere in Snowdonia this particular kind of rock bears a rich arctic–alpine flora which makes Cwm Idwal and other parts of the district of great botanical interest. The southern and northern portions of the wood, on a hard acidic rhyolite, present a striking contrast, having a grassy ground flora often associated with bracken and sometimes with short bilberry and mosses.

Also included within the reserve are the Ardda alder woodlands, which extend along the northern bank of the Afon Ddu and represent an extension of Coed Dolgarrog into the upland country beyond the steep valley slopes of the Conway. This section is at a higher altitude, being between 750 and approximately 850 ft., with a correspondingly higher annual rainfall—about 60–70 in. as compared with 50 in. for the main body of Coed Dolgarrog. The woods occupy a gently sloping upland valley, which is thickly covered with glacial drift. Soils of impeded drainage are widespread, hence the abundance of the alder and plant communities of wet soils. These woods have escaped clearing from the sixteenth century onwards because the valley in which they grow is extremely inaccessible and the pressure of land use has been correspondingly smaller throughout the centuries. They are of particular interest in the study of the alder at a relatively high altitude.

Permits will be necessary for those who wish to undertake research, to collect specimens, or to visit areas away from the rights of way. Applications for permits should be sent to the Regional Officer for North Wales, in Caernarvon.

Coed Gorswen Nature Reserve, lying at the northern end of the Conway Valley on the western side of the river near Rowen, is a good example of a lowland oak wood. The reserve, which covers 33 acres of the woodland and lies within the Snowdonia National Park, has been established under leases with the owners of the land.

The subsoil of Coed Gorswen is a very bouldery glacial drift and probably as a consequence of this it has been woodland for a very long time, perhaps throughout the historical period. The oaks consists of a heterogeneous population of intermediate forms between the sessile and pedunculate oak. As well as oak, other trees present in some quantity include elm, ash and alder. The ground flora is typical of rather base-rich soils, including such species as dog's mercury, sanicle, slender false-brome, common enchanter's night-shade, broad helliborine, skull cap and very locally yellow archangel. Fringing the wood is some interesting fenny ground—no doubt the "gors wen" (white marsh) which gave the wood its name—dominated by purple moor-grass with associates such as quaking grass.

Permits, which will be required to visit parts of the reserve away from the rights of way, to collect specimens of animals or plants, or to undertake research, can be obtained from the Regional Officer for North Wales.

Allt Rhyd-y-groes Nature Reserve is situated in the valley of the Afon Doethie, a tributary of the Towy, about nine miles north of Llandovery in Carmarthenshire. The reserve consists of 46 acres of oak woodland and has been leased to the Nature Conservancy by the Earl of Cawdor. It is hoped that it will ultimately be extended to include other areas of outstanding scientific interest in the locality.

The reserve overlies shales of Silurian age, and extends between 500 and 1,000 ft. on the west side of the steep flanks of the Doethie Valley. The woodlands are dominated by the sessile oak, as is the Coed Rheidol Nature Reserve in Cardiganshire, but here at Allt Rhyd-y-groes a number of the trees are well grown and display a vigour which is not often seen in equivalent woods elsewhere in Wales. The drier, more eroded slopes are clothed in sweet vernal grass together with mosses at the lower levels, and sheep's fescue higher up the slope.

Permits to visit parts of the reserve away from the rights of way, to collect specimens of animals or plants or to undertake research, can be obtained from the Regional Officer for South Wales, The Nature Conservancy, c/o Department of Zoology, University College of Swansea, Singleton Park, Swansea.

Blackcliff and Wyndcliff Forest Nature Reserve. An agreement has recently been reached between the Nature Conservancy and the Forestry Commission on the management of approximately 200 acres of Blackcliff and Wyndcliff Woods, forming part of the Commission's Tintern Forest, Monmouthshire, as a Forest Nature Reserve in the interest of nature conservation and ecological research jointly with those of timber production. The high amenity value of the area, which is close to Tintern Abbey and within the Forest of Dean Forest Park, will be maintained. These woods, situated on the Carboniferous limestone of the Wye Valley, have long been regarded by botanists as being of outstanding scientific interest; they were visited by John Ray in 1662 and by Sir Joseph Banks and John Lightfoot in 1773. The steep slopes are finely wooded with ash, wych elm, beech, small-leaved lime, yew, oak, cherry and whitebeam and service tree, together with a number of shrubs including dogwood, guelder rose and spurge laurel. The ground flora is rich and includes some locally uncommon species: lily of the valley is found there, and white angular Solomon's seal occurs on the cliffs.

Access will be unrestricted, but permits will be required to collect specimens of animals or plants, or to undertake research. Applications for permits should be sent to the Regional Officer for South Wales at the University College of Swansea.

INVESTMENT IN THE COAL, ELECTRICITY AND GAS INDUSTRIES

IN replying for the Government in a debate on fuel and power policy in the House of Commons on February 6, the Parliamentary Secretary to the Ministry of Power, Sir Ian Horobin, said that the oil companies and the Central Electricity Generating Board were considering together whether, within the framework of existing contracts, it would be practicable to postpone the conversion of two more power stations to oil during 1959; even in the changed industrial climate of the past twelve months Sir Ian said it would be wrong and improper to break contract or use pressure to that end. In moving the second reading of the Electricity (Borrowing Powers) Bill on January 20, the Paymaster-General, Mr. R. Maudling, said that the purpose of the Bill was to increase the authorized capital of the industry under the Bill, and the Electricity Council's permitted borrowings would be increased to £2,300 million, those of the North of Scotland Hydroelectric Board to £300 million and of the South of Scotland Electricity Board to £135 million. The total capital investment of the industry envisaged over the next seven years amounted to £2,130 million, of which £1,470 million would be in generation and £660 million in distribution: these figures were for the Electricity Council alone. This plan was based on estimated demand for electricity over the period, including a two-thirds increase in consumption, in which farm uses would be doubled and other commercial use would increase by about 50 per cent, or an annual increase of 6·6 per cent compared with 7·6 per cent in the past seven years. To meet this estimated increased demand of just under 9 million kilowatts, new plants for 12·25 kilowatts would be commissioned. The load factor had improved from 42·7 per cent in 1947 to 46·8 per cent in 1958 compared with a probable maximum of 60 per cent, and the problem of increasing the factor was being tackled with vigour. The cost per kilowatt

of increased capacity was now no higher than ten years ago, in spite of the increase in cost of materials and labour. As regards nuclear power, the cost of the Bradwell and Berkeley stations would be about three times that of a coal-fired station, but that of Hinkley Point should be about 2·5 times, and it was now calculated that this would be about £120 a kilowatt. By the nineteen-sixties the nuclear plant should be marginally more economical than conventional plant. There had already been a reduction at the rate of about 3 million tons of coal a year in the amount of oil to be used for power stations in the early nineteen-sixties, and the Government believed that the demand of the power stations for coal would rise throughout the next seven years.

Lord Mills, the Minister of Power, added a few further details in moving the second reading of the Bill in the House of Lords on March 3. Since some of the existing plant will not be fully operated, and because of obsolescence, the net increase in output capacity in England and Wales will be just over 10 million kilowatts, while the South of Scotland Electricity Board expects to commission more than 1 million kilowatts of new plant and the North of Scotland Hydroelectric Board about 460,000. The load factor is expected to increase to 49·2 per cent by 1964–65, and Lord Mills hoped that substantial improvements could be made in the methods of transmission and distribution so as to reduce the need for overhead lines in the countryside. The Boards had very much in mind the need to find means of diminishing the impact of their lines on amenities. Apart from the hydroelectric plant in the north of Scotland, practically all this new plant would be coal-fired or nuclear, and it was estimated that nuclear power could absorb nearly 47 per cent of the £1,100 million which the Central Electricity Board proposed to spend on power station construction. The nuclear power programme for 1964 onwards had not yet been decided, but apart from water-power we intended to rely in the long-term on coal and on nuclear power: less than 4 per cent of the investment in power stations proposed over the next seven years represented oil-fired plant.

These statements form a background to the third White Paper* on capital investment in the coal, electricity and gas industries, which is limited to the years 1957–59. Actual expenditure in 1957 was £434·7 million, compared with £438·9 million and £524·7 million approved for 1958 and 1959, respectively. Of these figures, £240·2 million, £241·3 million and £303·5 million are for the Electricity Council and Boards in the respective years: actual expenditure in 1958–59 is estimated to have been £258·5 million, of which £106·5 million was for conventional power stations and £38·0 million for nuclear power stations. The corresponding figures in the approved 1959–60 programme are £117·5 million and £57·0 million, and payments in respect of the nuclear power stations at Bradwell, Berkeley and Hinkley Point will reach their peak during the year. The North of Scotland Hydroelectric Board's programme of capital expenditure of £10 million on generation for 1959 is mainly for hydroelectric schemes at present in hand, while that of the South of Scotland Electricity Board of £16·2 million on generation in 1959 is almost entirely accounted for by the nuclear power station at Hunterston and the coal-fired station at Kincardine.

* Capital Investment in the Coal, Electricity and Gas Industries. Pp. 8. (Cmnd. 713.) (London: H.M. Stationery Office, 1959.) 6d. net.

BATHYSCAPHE *F.N.R.S.* III

ONE of the greatest recent advances in methods of deep-sea research is that of direct observation under water by photography, television and the human eye. The latter had already been shown possible before the Second World War by Otis Barton and William Beebe in the cable-dependent bathysphere.

In 1939, Prof. Auguste Piccard and Prof. Max Cosyns gained the support of the Fonds National de la Recherche Scientifique Belge (F.N.R.S.) to build their free-navigating bathyscaphe, but owing to the War its construction was delayed. The first tests were made in December 1948 off Dakar in the vessel which was entitled *F.N.R.S.* II, the Piccard–Cosyns stratosphere balloon being *F.N.R.S.* I. A depth of 1,380 m. was achieved without passengers. Although improvements were needed, the possibilities of the bathyscaphe were proved, and in October 1950 an agreement was signed between the Belgian Fonds National de la Recherche Scientifique, the French Navy, and the French Centre National de la Recherche Scientifique for the construction by the French Navy at Toulon of *F.N.R.S.* III, using the sphere of bathyscaphe *F.N.R.S.* II. This new vessel was put into the water in June 1953 and Capitaine de corvette G. Houot was placed in command with Ingénieur du génie maritime P. Willm to assist. Together they carried out a series of tests ending off Dakar in February 1954 when they reached a depth of 4,050 m.

In May 1954 the French Centre National de la Recherche Scientifique obtained permission from the Navy to equip and use *F.N.R.S.* III for scientific purposes. A management committee was appointed under the stimulating presidency of Prof. Louis Fage. The first dive was made on April 18, 1954, at a position 25 miles south-west of Dakar to a depth of 850 m. with Prof. Th. Monod as the scientist on board; this was quickly followed by a dive to 1,400 m. Thereafter a certain number of dives have been made each year with Commandant Houot on board, accompanied by a qualified naturalist. By the end of 1957, twenty-eight dives had been made to depths between 130 m. and 2,290 m., eighteen of which were deeper than 1,000 m. Eight distinguished and courageous biologists took part. Their names are given here, together with the number of dives each made, because they should be remembered as pioneers of this new era of marine research: Prof. Th. Monod, Dakar (2); Prof. J.-M. Péres, Marseilles (9); J. Picard, Marseilles (4); Prof. Fr. Bernard, Algiers (3); J. Furnestin, Paris (1); Prof. Dubuisson, Liège (1); G. Trégouboff, Villefranche (6); M. Ruivo, Lisbon (1).

Dives were made in the Mediterranean and the Atlantic. *F.N.R.S.* III is equipped with apparatus for recording at any time the depth, temperature and pressure; it has equipment for collecting water samples with contained microplankton; and it has powerful light projectors and automatic cameras. It

does not yet possess means for efficient capture of larger planktonic or bottom-living animals, but observations can be supplemented by collections made by the accompanying vessel with pelagic nets and trawls.

A number of individual accounts have already been published, but a general review of the principal results obtained up to the end of 1957 has now been made available*. Since much of the biological information must be obtained by direct observation, it is essential that the accompanying biologist should be experienced, and that he should have a thorough knowledge of the fauna he is to investigate so that he may be able to identify as many organisms as possible. The more dives he makes also the more competent will he become at observing. Given these requirements there is no doubt that the results obtained will be of great value since the depth, temperature and light conditions can be known exactly. Indeed, most interesting information is already forthcoming on the distribution in depth and abundance of some plankton animals. The occurrence of coccolithophores in considerable numbers at great depths has also been established. A noteworthy feature is the abundance of particles in suspension at different depths. It is now known that the subsistence of the bottom sediments is reasonably firm. Numerous small mounds on the sea bottom are common, but prolonged watching has so far revealed no occupants. Unusual habits and movements of deep-sea fish have been observed. The presence of slow currents has been shown by particles drifting along the bottom.

There is no doubt that the value of the bathyscaphe as a tool in marine research has been proved by French scientists, to whom all honour is due; and as a result new models to attempt greater depths are on the way.

F. S. Russell

* Annales de l'Institut Océanographique fondées par S.A.S. Albert 1er Prince de Monaco. Publiées sous la direction de Louis Fage. Nouvelle Série. Tome 35, Fasc. 4 et dernier: Résultats Scientifiques des Campagnes du Bathyscaphe *F.N.R.S.* III, 1954–1957. Pp. 235–341+planches 17–24. (Paris: Masson et Cie., 1958.) 1,500 francs.

ALFVÉN WAVES IN A GAS DISCHARGE

By D. F. JEPHCOTT*

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In 1942 Alfvén[1,2] predicted the possible existence of magnetohydrodynamic waves, which may be regarded in the simplest case as transverse oscillations of magnetic field lines carrying with them a surrounding inviscid perfectly conducting fluid.

The velocity of propagation is given by:

$$V^2 = \frac{\mu H^2}{4\pi n M}$$

where H = magnetic field intensity; n = number of particles/cm.^{3}; M = mass of particle.

Alfvén's theory strictly applies only to waves in an incompressible fluid, but it can be extended to a compressible fluid (for example, an ionized gas) for the case of waves of small amplitude. A further restriction is that the frequency should be small compared with the ion cyclotron frequency[3].

Previous experimental work has been carried out in liquid conductors[4–6], and standing Alfvén waves have been reported in some gas discharges[7,8].

Our experiments have been carried out using toroidal gas discharges of 10 k.amp., and 200 μsec. duration, in monatomic gases at initial pressures of about 100μ, and with axial (B_z) fields in the range 3–14 k.gauss. Under these conditions, the current density was nearly constant across the area of the torus (except close to the walls) and the discharge sufficiently quiescent for satisfactory signals to be obtained from magnetic probes.

Fig. 1 illustrates the method used for exciting the waves and measuring their velocity.

When the gas current had reached its peak value, the condenser C was discharged via the spark gap S through the exciter conductors A and B, which were close to the outer wall of the torus. The condenser circuit oscillated at 260 kc./s., imposing perpendicular components on the main field, and resulting in torsional oscillations about the circular axis of the torus.

Oscillations of the order of 10 gauss in amplitude were picked up by the magnetic probe 2 and were found to be delayed relative to the signal from probe 1 which recorded the direct exciting field. No oscillations were observed with $B_z = 0$, and the amplitudes always increased with B_z over the working range. Velocities were calculated after a small correction had been made for the measured time-lag of the perturbing field at D ($d = 0$) in the plasma, relative to the direct vacuum field of the conductors.

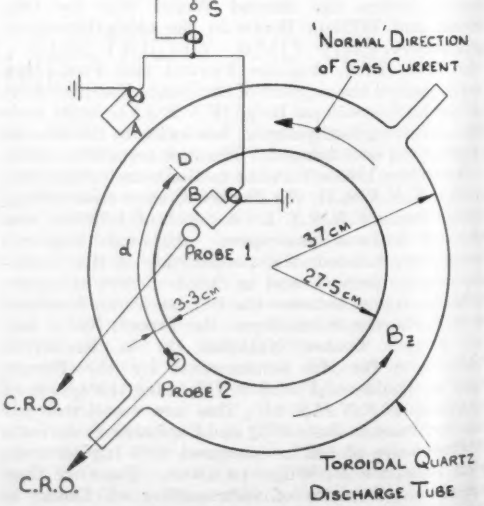

Fig. 1

* On attachment from Hawker Siddeley Nuclear Power Co.

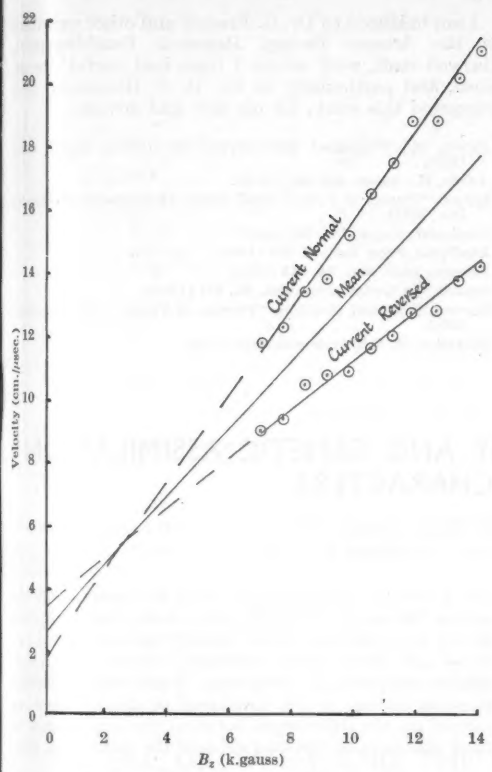

Fig. 2. Argon, 70μ; 10 k.amp.; d = 44 cm.

The velocities have been measured over a range of B_z values, keeping the gas current and initial pressure constant. For argon and xenon, the upper limit to B_z was set by the maximum field available, while in helium the signals became distorted at 10 k.gauss. The lower limit on B_z was set by the value below which the signals were too small to be measured accurately. In xenon, no readable signals were observed with d = 44 cm., and with d = 20 cm. the velocity increased with B_z only for fields greater than 10 k.gauss.

The exciter frequency was well below the ion cyclotron frequency in helium, just below it in argon, and above it in xenon (which probably accounts for the results obtained in xenon).

Velocities were measured with the gas current 'normal' (that is, flowing in the same direction as the waves being propagated from the exciter along the shorter arc to the gas probe) and 'reversed'. In all cases, the measured velocities with gas current normal were found to be higher than with the current reversed, the discrepancy increasing with B_z in the case of argon (see Figs. 2 and 3).

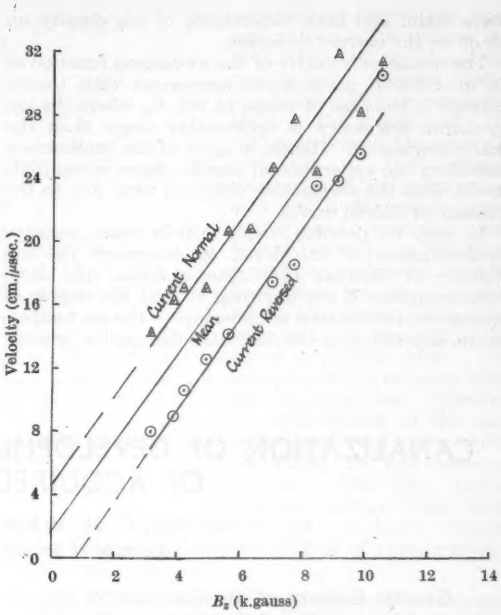

Fig. 3. Helium, 130μ; 10 k.amp.; d = 44 cm.

A theoretical treatment[9], involving the interaction of the axial current with the perturbed field, shows that some dependence of the velocity on current direction is to be expected, but at present the numerical agreement with experimental results is correct only in order of magnitude.

For the present, we assume the wave velocity to be given by the mean of the velocities measured with the current normal and current reversed.

The positive intercepts on the velocity axes in Figs. 2 and 3 may be due to two possible causes:

(1) No correction has been made for any increase in wave velocity due to damping.

(2) Since the axial magnetic field along any arc is inversely proportional to the radius of the arc, while d is directly proportional to the radius, waves being propagated close to the outer wall tend to lag behind those near the inner wall. Hence, if there is any coupling between waves travelling along arcs of different radii, the wave velocity may be some mean value of the local Alfvén speeds.

Table 1 gives a summary of the results obtained so far in helium and argon. From the Alfvén velocity expression, the ion density has been calculated as given by the slope of the graph of mean velocity against B_z. For argon, the ion density has also been checked with a Langmuir double probe, and the two values for ion density thus obtained show satisfactory agreement. The double probe signals also indicated no changes in ion density due to the passage of the

Table 1

Gas	Gas current (k.amp.)	B_z range (k.gauss)	Range of Alfvén wave-lengths (cm.)	Ion cyclotron frequency range (kc./s.)	Exciter frequency (kc./s.)	Particle density before discharge (No./cm.^{3})	Ion density from wave velocity (No./cm.^{3})	Ion density from double probe (No./cm.^{3})	Electron temperature from double probe (deg. K.)
Helium	10	3–10·5	40–120	$1{\cdot}14 \times 10^3$–$4{\cdot}0 \times 10^3$	260	$4{\cdot}6 \times 10^{15}$	$1{\cdot}51 \times 10^{15}$	—	—
Argon	10	7–14	40–70	270–540	260	$2{\cdot}5 \times 10^{15}$	$1{\cdot}04 \times 10^{15}$	$1{\cdot}14 \times 10^{15}$	$4{\cdot}7 \times 10^4$

wave train, and little dependence of ion density on B_z or on the current direction.

The measured velocity of the waves as a function of B_z in different gases shows agreement with theory (except in the case of xenon at low B_z, where the ion cyclotron frequency is appreciably lower than the wave frequency). Hence, in spite of the preliminary nature of the experimental results, there seems little doubt that the oscillations observed were due to the passage of Alfvén waves.

It may be possible, using Alfvén wave velocity measurements of this kind, to determine the ion density of plasmas in magnetic fields, and from measurements of the damping to find the degree of ionization, the electron temperature or the ion temperature, depending on the dominant dissipative process.

I am indebted to Dr. G. Francis and other members of the Atomic Energy Research Establishment, Harwell staff, with whom I have had useful discussions, and particularly to Dr. R. J. Bickerton, who suggested this work, for his help and advice.

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CANALIZATION OF DEVELOPMENT AND GENETIC ASSIMILATION OF ACQUIRED CHARACTERS

By Prof. C. H. WADDINGTON, C.B.E., F.R.S.
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Genetic Control of the Canalization of Development

In multi-cellular organisms the development of tissues and organs commonly exhibits a balance between flexibility and inflexibility; while a sufficient external stress will cause development to divergence and reach some unusual end-result, there are counteracting tendencies to attain the normal adult condition even in the face of disturbing influences. This condition, which has been referred to as the 'canalization of development'[1], must be regarded as a result of the complex interactions between the numerous gene-controlled processes by which development is brought about. It should therefore be possible to modify the degree of flexibility–inflexibility by selection of appropriate genotypes. There has as yet been little direct evidence on this point, although it has been shown that there is some control by the genotype over the repeatability of development (the inverse of developmental 'noise')[2].

Many mutant phenotypes, in *Drosophila* and other organisms, are notoriously sensitive to environmental influences. Experiments were carried out with, among other phenotypes, that of Bar in *D. melanogaster*. The number of facets in the eye is much reduced, but is larger at low temperatures than at high. The offspring of a number of pair matings were divided into two lots, one of which was grown at 25° C., the other at 18° C. Selection was made by breeding from the families with the smallest differences between the family means at the two temperatures. After only five generations, the difference was reduced from about 100 to around 12 (Table 1). The lowering of the difference has involved both an increase in the size of the eye at the high, and a reduction of its size at the low, temperatures; clearly the selected genotypes are much more inflexible (better canalized) against temperature variations. There was no accompanying change in the 'noisiness' of development, as judged by the differences between the logarithms of facet numbers on the two sides of the individual flies.

Table 1. Bar. Facet Numbers in Females in Unselected Controls, and in three Families resulting from Selection

	No. of families	Average family mean, at 18° C., and S.E.	Average family mean, at 25° C., and S.E.	Difference, family means 18° C. and 25° C.
Unselected	9	156·25 ± 4·81	55·46 ± 0·81	100·80 ± 4·52
Selected 6a	8	106·02 ± 1·17	96·52 ± 1·67	14·50 ± 2·29
Selected 6b	7	100·76 ± 1·38	95·30 ± 1·70	5·46 ± 1·98
Selected 6c	8	111·39 ± 1·72	98·48 ± 2·67	12·92 ± 3·05

Genetic Assimilation of an Adaptive Acquired Character

It has been shown that if a strain of organisms is selected for several generations for increased readiness to become developmentally modified in some specific manner in response to an environmental stress, the later generations may exhibit the modification even in the absence of the stress which was initially required to produce it; and it has been argued that this process of 'genetic assimilation' may play an important part in the evolution of adaptive characters[1]. The experiments which have been described so far have dealt with characters which (*a*) would not be adaptive in Nature, and (*b*) the production of which involved threshold phenomena. Both these limitations have been removed in a recent experiment.

Three strains of *Drosophila melanogaster* (Oregon wild-type, sp^2bs^2 and *al b c* sp^2) were grown on normal medium to which sodium chloride had been added in sufficient quantities to lead to considerable mortality (> 60 per cent) of the larvæ. Thus, stringent natural selection occurred; and by increasing the salt concentration in later generations, the intensity of selection was maintained at a roughly constant level for twenty-one generations. At that stage counted numbers of eggs were set up on media of various salt concentrations, and the unselected stocks from which the experiment had started were tested simultaneously. The percentage of survivors was ascertained on each medium, and measurements were also made of the area of the anal papillæ, since it has been suggested by Gloor and Chen[3] that these organs are concerned with the osmotic regulation of the larva, although their exact function is not well understood.

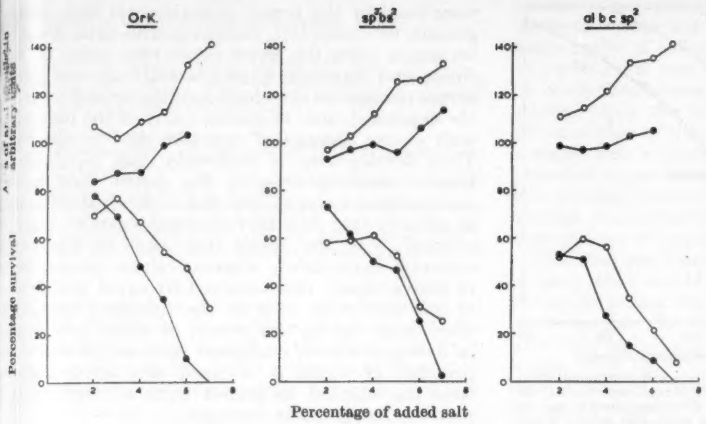

Fig. 1. Percentage survival (below) and size of anal papillæ (above) in selected and unselected strains in various concentrations of salt.
Unselected, ●—●; selected, ○—○

Measurements of the papilla area were made on young prepupæ to minimize alterations due to the contractility of the larva.

As can be seen from the lower graphs in Fig. 1, the selected strains survive better than the unselected at the high salt concentrations, which range up to 7 per cent—twice as high as sea water. From the upper curves, it is clear that the area of the anal papillæ increases in higher salt concentrations. Two points are noticeable: (1) the size of the papilla in the selected strains is greater at all concentrations than in the unselected; (2) the steepness of the curve is increased in the selected strains. The first point indicates that some genetic assimilation has occurred; the selected strains in a relatively normal environment (low salt) have anal papillæ of a size which the unselected strains only attain in response to the stress of added salt. The second point is evidence that the selection has improved the adaptability of the animals, rendering them more responsive to salt concentration. The first point, however, shows that their adaptability is still limited, and it is clearly because of this limitation that some degree of genetic assimilation has occurred.

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CELL GROWTH AND THE DEVELOPMENT OF TUMOURS

By ANNA KANE LAIRD and A. D. BARTON
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IN the course of biochemical studies[1,2] of liver cell fractions carried out during the induction of liver tumours by known carcinogens, a fairly regular pattern of cell proliferation became apparent. It is the purpose of the present article to relate this pattern to earlier observations by other investigators, and to discuss various implications concerning the carcinogenic process.

In studies in which liver tumours were induced in rats by continuous administration of diets containing 2-acetylaminofluorene[3] or 4-dimethylaminoazobenzene[4], others have shown that grossly recognizable tumours are not obtained until 200 or more days after the animals are placed on the carcinogenic diet. These workers have also shown that it is not necessary that the administration of the carcinogen be continued until tumours are visible; it must be continued only through a certain critical period. For example, if the animals are returned to a normal diet after only 25–30 days exposure to 2-acetylaminofluorene or 4-dimethylaminoazobenzene, few if any will develop tumours; if they are exposed for a total of 50–75 days, a large proportion of the rats will develop tumours, and the number is not greatly increased by continuing the administration beyond this time. This finding suggests two conclusions concerning the neoplastic transformation that ultimately leads to the production of a tumour: (1) malignant cells are not produced unless administration of the carcinogen is continued beyond the time required to produce a significant threshold effect, the nature of which is undefined; (2) when the transformation does occur, it takes place within a relatively short period of time.

In the present experiments, male rats were fed continuously a diet containing 2-acetylaminofluorene. Two such experiments were carried out, using rats of different strains and different initial body-weights (details are given in the legend to Fig. 2). The changes in weights of body and liver obtained in the second experiment are shown in Fig. 1 (the corresponding results in the first experiment were similar). Within a few days of the inception of the carcinogenic treat-

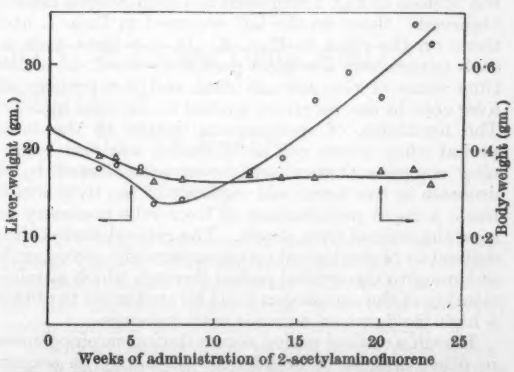

Fig. 1. Changes in weights of liver and body of rats during administration of 2-acetylaminofluorene in the diet, at a concentration of 0·06 per cent

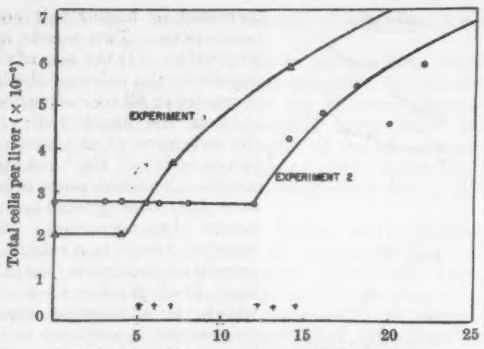

Fig. 2. Changes in the total number of liver cells during administration of 2-acetylaminofluorene, in two different experiments. In Exp. 1, rats of the Holtzman strain, weighing 180–200 gm. at the start of the experiment, were fed a grain diet mixed in the laboratory (ref. 2); the food contained 2-acetylaminofluorene in an initial concentration of 0·05 per cent, which was later reduced to 0·04 per cent. In Exp. 2, rats of the Sprague–Dawley strain, weighing 350–450 gm., were fed a ground 'Purina' diet containing 2-acetylaminofluorene in a concentration of 0·06 per cent. The crosses at the bottom of the graph represent spontaneous deaths of individual rats; the left group of four occurred in Exp. 1, and the right group of three occurred in Exp. 2

ment, the weight of the body fell, until it levelled off at a value distinctly below the initial weight (and even further below the weight of normal controls the weight of which had been increasing in the meantime). In contrast to the fall in weight of the body, that of the liver increased continuously to well above the control values, following a relatively brief initial fall.

The total number of cells in such livers may be determined by introducing appropriate dilutions of a homogenate into a hæmacytometer and counting the cell nuclei present. Since each homogenate is prepared from fresh liver pulp, from which a major part of the connective tissue is removed by use of a tissue press, the cells counted are mainly parenchymal. The changes in the total number of cells per liver are shown in Fig. 2. The course of the changes ran closely parallel in the two experiments. After a period in which no net change in the average number of liver cells was observed, a rapid increase occurred, the rate of which slowed as the carcinogenic period progressed. The increase in number of cells began later in the experiment in which the larger rats were used; the reason for this difference is not understood, but is under investigation. The crosses at the bottom of Fig. 2 represent the spontaneous deaths observed; those on the left occurred in Exp. 1, and those on the right in Exp. 2. It is evident that in each experiment a critical period occurred, at which time some of the animals died and the number of liver cells in the survivors started to increase rapidly. The limitation of spontaneous deaths to the brief period when active cell proliferation was first beginning suggests that a crisis was precipitated by a decrease in the functional capacity of the liver which made a rapid proliferation of liver cells necessary to save the animal from death. The critical period thus defined in physiological terms apparently corresponds in timing to the critical period through which administration of the carcinogen must be continued to obtain a high incidence of animals with tumours.

If such a critical period occurs during carcinogenesis in other organs, it would not necessarily be accompanied by an increase in mortality, if the target organ were one not required for survival, or if destruction were local in the organ (experimental skin carcinogenesis, for example). Survivors who have developed leukæmia since the atom bomb explosions in Hiroshima and Nagasaki quite generally showed signs of severe damage to the blood-forming organs soon after the exposure[5], and, of course, many of the individuals with severe damage of this sort died at that time. Thus development of leukæmia may be similar to hepatic carcinogenesis, to the extent that in both cases a tissue or organ essential to survival is damaged so severely that its total functional capacity is greatly reduced, with the result that some of the victims succumb immediately whereas others enter a period of active repair, characterized by rapid proliferation of the surviving cells in the damaged tissue. In some cases during this period of active proliferation in damaged tissue, malignant cells are produced, the presence of which is revealed only after sufficient time has elapsed to permit their multiplication to produce recognizable tumours.

When instead of 2-acetylaminofluorene, rats were fed 3′-methyl-4-dimethylaminoazobenzene, an azo dye and a well-known hepatic carcinogen, the changes in number of cells shown in Fig. 3 were observed. The time-scale is much expanded compared with Fig. 2, and the curve therefore constitutes essentially a magnification of the early changes seen in the two curves of Fig. 2. Although the carcinogen used in this experiment differs from 2-acetylaminofluorene both chemically and in its pathological effects, it produced very similar changes in the total number of liver cells: an initial period without change in number of cells, followed by an abrupt increase in the number of liver cells. In this case, the number of cells in the liver increased by 50 per cent in two days.

Since the tumour cells occur in discrete nodules which grow separately from the rest of the tissue, and since numerous mitotic figures are found within the tumour mass, it seems likely that growth of the tumour occurs not by continued transformation of liver cells into tumour cells, but rather by proliferation of the tumour cells themselves. It is probable that each discrete tumour arises from one malignant cell. If we consider the actual number of cells involved in the sudden 50 per cent increase noted in the experiment with 3′-methyl-4-dimethylaminoazobenzene described in the preceding paragraph, we find that 10^9 new cells were formed in 48 hr. If this is the case, then to produce 100 tumours, a malignant change would have to occur in only one cell per ten million new cells formed. Even if only 1 per cent of the cells undergoing a malignant transformation

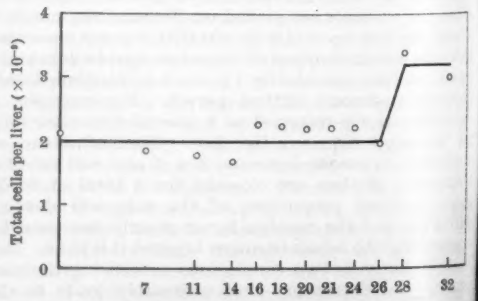

Fig. 3. Changes in the total number of liver cells during administration of 3′-methyl-4-dimethylaminoazobenzene. Note that the time-scale is much expanded compared to Figs. 1 and 2

Table 1

Given: Weight of tumour-bearing livers, approx. 35 gm.
Weight of tumours, approx. 4 gm.
Cell count, per gm. liver, approx. 200×10^6
Cell count, per gm. tumour, approx. 450×10^6
Then: Tumour-bearing liver contains about $8,000 \times 10^6$ cells, of which about $1,800 \times 10^6$ cells are tumour cells and about $6,200 \times 10^6$ cells are liver cells.

If 4 gm. of tumour constitute:
(1) All one tumour mass, all from one cell: then 31 cell generations would be required.
(2) 4 separate tumours, 1 gm. each, each from one cell: then 29 cell generations would be required.
(3) 4,000 separate tumours, each 1 mgm., each from one cell: then 19 cell generations would be required.
But the liver started with $2,000 \times 10^6$ cells, and contained $6,200 \times 10^6$ at the end of the experiment: this would require less than 2 cell generations.

survived to produce a tumour, the incidence of malignant change in the above example would still be only one cell in one hundred thousand. In other words, even during continued exposure of liver cells to a carcinogen, the carcinogenic change is probably a very rare event.

In Table 1 are given the data necessary for calculating approximately the number of cell generations required to produce the tumours observed, and also the number of cell generations occurring in the surrounding liver tissue, assuming that all daughter cells survive in both cases. The figures given for weights of liver and tumour are representative of our findings with 2-acetylaminofluorene. The tumour data have been calculated for three conditions: (1) the tumour tissue occurs in the form of a single tumour mass; (2) the tumour tissue is found as several 1-gm. tumours; and (3) the tumour tissue occurs in the form of several thousand 1-mgm. tumours. The condition observed in practice most closely approximates the second case, with tumour nodules ranging in number from less than 10 to about 100. The data in Table 1 show that during the period in which the tumour cells have passed through 20–30 cell generations, the surrounding parenchymal liver tissue has passed through less than two cell generations on the average. Without radioautography, it is difficult to know whether such average increases in cell number are due to a few divisions of many cells, or to many divisions of a few cells. However, if the data of Fig. 3, obtained with 3′-methyl-4-dimethylaminoazobenzene, can be carried over to the experiments with 2-acetylaminofluorene, then the increase in cell number observed early in the experiment is due principally to a few divisions of a large number of liver cells, since the 50 per cent increase in number of cells observed in that experiment occurred within too brief a period of time to permit many cycles of cell division to be repeated. It might be pointed out here that in the very rapid cell multiplication that occurs in liver regenerating after partial hepatectomy, the total increase in number of cells also amounts to less than two cell generations on the average, and since such an increase in cell number is preceded by an increase of more than 50 per cent[6] in the average content of deoxyribonucleic acid per nucleus, it is probable that here, too, the increase in mass of liver is due to a few divisions of many cells.

If at first less than one cell in a million is producing progeny that would eventually yield tumours, then the rapid initial rise in the total number of cells in the livers must be due primarily to multiplication of liver cells rather than potential tumour cells. If this is true, then much of the mitotic activity of the liver cells is already completed soon after the initial burst becomes evident. Comparison with the earlier data[3,4] suggests that this initial burst of cell division is the essential feature of the critical period through which the administration of the carcinogen must be continued in order to obtain a high incidence of animals with tumours. Unless the administration of the carcinogen is maintained until the burst of cell division is produced, few, if any, malignant cells are produced. Once this burst of cell division is elicited, sufficient malignant cells are produced to make further administration of the carcinogen unnecessary. Since the critical period is so short, it is evident that the change from a normal liver cell to a malignant cell can occur within a short time, possibly within one or two cell generations. This suggests that the change essential to malignancy is sudden and complete, rather than continuous over many cell generations. Further important changes in the direction of increasing anaplasia and independence of normal growth-control mechanisms are known to occur[7], but we are considering here only the initial, essential step.

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GEL FILTRATION: A METHOD FOR DESALTING AND GROUP SEPARATION

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WE wish to report a simple and rapid method for the fractionation of water-soluble substances. The method is based on a column procedure similar to chromatography in which the stationary phase is comprised of a new type of gel. These gels consist of hydrophilic chains which are cross-linked. They are devoid of ionic groups, the polar character being almost entirely due to the high content of hydroxyl groups. While water-insoluble, the gels are nevertheless capable of considerable swelling. The fractionation depends primarily on differences in molecular size although phenomena have been observed which indicate the influence of other factors.

The idea that gels might be used for the zone separation of substances differing in molecular size is not a new one. Synge and Tiselius[1], for example, have

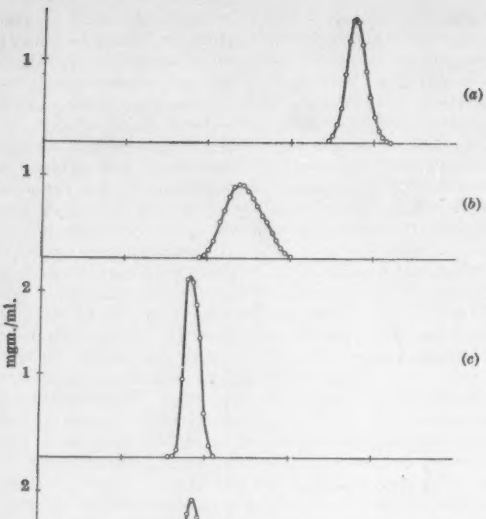

Fig. 1. The diagrams illustrate 'molecular sieving'. In experiments *a*, *b* and *c*, 5 ml. of 1 per cent aqueous solutions of glucose and dextran fractions of $\bar{M}_n$ 1,000 and 20,000, respectively, were filtered through a dextran gel column of the size 4·0 × 36·5 cm. Diagram 1*d* shows the result of a similar experiment with a mixture of the substances mentioned

suggested such a method, based on the effect of friction during electro-osmotic migration through gels. In a homologous series of uncharged substances the friction may be expected to rise with increasing molecular weight. Thus the higher homologues should be retarded more strongly than the lower ones. This is, in fact, the case as was experimentally confirmed by Mould and Synge[2].

On filtration through a bed of starch particles, high and low molecular weight substances also behave differently. In this case, however, it is the lower molecular weight substances which are retarded. We frequently encountered this type of phenomenon when using starch as a supporting medium in zone electrophoresis[3]. It appears likely that the cause of this behaviour is a restricted diffusion of the kind observed by Biget[4]. The restriction is related to the effect of friction observed when solutes are electro-osmotically transported through gels.

Lathe and Ruthven's meticulous and thorough investigation[5] gives a good conception of the possibilities which are inherent in starch filtration. However, starch itself has several undesirable properties. Among these are instability, poorly defined composition and the high resistance to flow of starch columns. Together, these properties have caused the method to be of little practical importance so far.

The above disadvantages seem to be eliminated in the case of certain synthetic gels such as dextran gel. The latter is a material originally synthesized in an attempt to obtain an ideal convection depressor[6] for preparative zone electrophoresis. It is prepared by cross-linking dextran in such a way that the polysaccharide chains form a macromolecular network of great stability. A bed of particulate water-swollen dextran gel has very interesting properties. Aqueous solutions filter rapidly through it at ordinary pressures, and no significant quantity of the gel material is dissolved whether in alkaline, neutral or acid medium. Macromolecules are completely excluded from the gel phase and migrate without retention in the interstitial fluid. However, substances of low or intermediate molecular weight penetrate the gel particles to an extent which is in most cases determined by their molecular dimensions and the degree of cross-linkage of the gel. The separating ability of columns packed with dextran gels is based on these facts.

We have studied dextran gels ('Sephadex' obtained from Pharmacia, Uppsala, Sweden) with varying degrees of cross-linkage graded on the basis of water regain[8], and also other hydrophilic gels derived, for example, from starch and polyvinyl alcohol. Like dextran gels, the latter are chemically stable and as a rule have satisfactory mechanical properties. Moreover their separation properties may be varied within wide limits. More detailed reports will be published later. A few simple examples of the use of the method will be given here.

Some experiments with dextran fractions[7] and glucose are shown in Fig. 1. A glass tube with an inner diameter of 4·0 cm. was packed with 100 gm. of dextran gel suspended in distilled water. The final height of the bed was 36·5 cm. The particle size of the dried form of the gel was between 100 and 200 mesh and its water regain was 2·5 gm. per gm. of dry weight[8]. A filter paper was placed above the flat surface of the bed to prevent disturbances. The test solution was applied and when it had completely entered the bed the column was washed with distilled water at a constant rate of 110 ml./hr. The carbohydrate content was determined using the anthrone method. The recoveries in experiments 1*a*, 1*b*, 1*c* and 1*d* were 103, 95, 101 and 97 per cent, respectively. The elution volume of the substances and their distribution are independent of whether the substances are filtered through the columns singly or together. The oligosaccharide fraction has a wider distribution than the other substances due to poly-

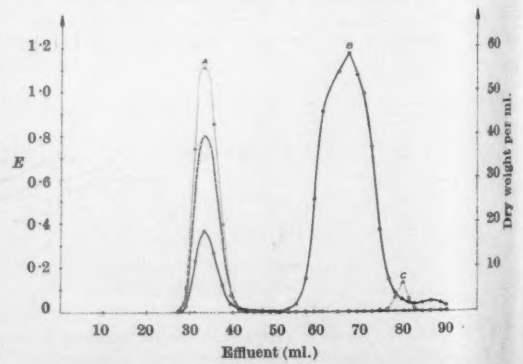

Fig. 2. Diagram showing the separation of ammonium sulphate from serum proteins as obtained after filtration of the solution of the mixture through a dextran gel column (2·5 × 17·5 cm.). Fractions (2 ml. each) were collected and analysed by the method of Folin–Lowry (0·01 ml. aliquots, — × —) and by ultra-violet light absorption at 280 mμ (— ○ —). The weight distribution of the substances in the effluent (— ● —) was determined by weighing 1-ml. aliquots after evaporation *in vacuo*

dispersity. On filtration it is fractionated, and molecular weight determinations of the material from different parts of the peak indicate that the higher homologues are located at the beginning of the peak.

An experiment with serum proteins performed in order to demonstrate desalting is shown in Fig. 2. 5 ml. of a saturated ammonium sulphate solution was added to 5 ml. of fresh human serum and the precipitate was removed by centrifugation. 3 ml. of the supernatant was applied to the top of a bed (2·5 × 17·5 cm.) of the same type of dextran gel as in the previous experiment. The substances were eluted with distilled water at a constant rate of 25 ml./hr. A qualitative analysis for sulphate ions in peak *A* was negative, showing that the proteins had been freed effectively from salt. No proteins could be demonstrated in peak *B*, which contains the entire amount of ammonium sulphate. Peak *C* contains a low molecular weight substance with an absorption maximum at 292 mμ. The yield of material absorbing in ultra-violet at 280 mμ was 102 per cent, that is, it was quantitative within the limits of error.

The result of a gel filtration is often similar to that obtained by dialysis. The unavoidable dilution is naturally a disadvantage but it may be kept within reasonable limits by a suitable choice of experimental conditions. It should be mentioned that the rate of elution and the concentration of solutes can be varied greatly without causing a serious spreading of the zones or altering the position of the peaks. It is necessary in each problem of separation to compromise with respect to those factors which condition the separation efficiency and the rate of elution.

As a desalting method gel filtration presents many advantages relative to dialysis. For example, we have never observed complications corresponding to the clogging of dialysis membranes. The method is especially suited for changing a protein solution from one buffer system to another, a step which is often necessary prior to ion exchange chromatography and electrophoresis.

Gel filtration may be performed with great rapidity and nevertheless causes the complete separation of two or more substances or classes of substances if the differences in molecular size are sufficient. The scale of a procedure such as the desalting of a protein solution may be increased upwards without difficulty so that considerable quantities may be handled.

Since after each complete operation the gel is automatically regenerated, gel filtration is well suited for cyclic purification procedures.

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OCCURRENCE OF 'ESTER-LIKE' LINKAGES IN COLLAGEN

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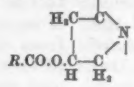
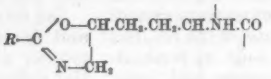
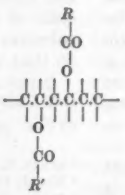
MANY investigators have considered the possibility that primary linkages other than peptide or iminopeptide bonds contribute to the stability of the collagen structure. Thus, Gustavson[1] postulated the existence of ester linkages between the hydroxy groups of the abundant hydroxyamino-acid residues of collagen and the carboxyl groups of terminal residues or of dicarboxylic acid residues elsewhere in the protein. Ames[2] discussed the possible occurrence of an imide cross-linking bond of the $R.C = O.NH.C = O.R'$ type. In Table 1 are listed some of the primary linkages other than peptide which are possible for collagen. Except for the diester compounds, linkages to carbohydrates have not been included, since the small amount of carbohydrate found in purified collagens could not account for the extent of the hydroxylamine reaction as described here. Except for the side-chain amide and guanidine groups, cleavage of the linkages listed in Table 1 could result in significant decreases in the molecular weight of collagen, and, except for the additional case of interchain peptide bonds, in the production of defined polypeptide units or the separation of individual chains. The presence of acid anhydride bonds to account for the experimental findings is not likely when one considers the conditions under which commercial gelatins are prepared.

It is known that esters may be differentiated from amides or peptides because of their much faster rates of reaction with hydroxylamine at *p*H values of 8–11, and that imides of the type shown in Table 1 may be as reactive as esters. Williams *et al.*[3] showed that certain γ-glutamyl peptides were sensitive to treatment with aqueous hydroxylamine, and Braunitzer[4] claimed that a cyclic iminopeptide linking the β-carboxyl group of an aspartic acid residue and the imino group of a proline residue was cleaved by relatively mild treatment with hydroxylamine. The latter observation led Braunitzer to conclude that an N-terminal proline group of tobacco mosaic virus protein is masked by such a combination, a view which Narita[5] could not confirm.

In the experiments described here, at least three types of gelatin were used: commercial acid- and base-processed gelatins, and gelatins prepared from calf-skin and carp swim-bladder procollagens by mild heating in water or by denaturation with 6 *M* urea. Treatment with hydroxylamine was performed either at room temperature or at 40° C.; in the former case 6 *M* urea was present during the reaction to prevent gelation. Solutions of gelatin containing 2–4 per cent protein were mixed with equal volumes of 2 *M* hydroxylamine hydrochloride solution previously adjusted to *p*H of approximately 10 with sodium hydroxide. In kinetic studies, aliquots were

Table 1. Possible Hydroxylamine-sensitive Bonds in Collagen

Type linkage	Structure	Residues which may be involved*
Side-chain amide	$R.CO.NH_2$	$GluNH_2$ or $AspNH_2$
Inter-chain imide	$R.CO.NH.CO.R'$	$GluNH_2$ and Asp or $AspNH_2$ and Glu
Side-chain guanidyl group	$R.NH.C(:NH)NH_2$	Arg
Inter-chain acyl-guanidine link	$R.NH.C(:NH)NH.CO.R'$	Arg and Asp or Arg and Glu
Inter-chain ε-lysyl peptide	$R.CO.NH.CH_2.R'$	Lys and Asp or Lys and Glu
Inter-chain ester	$R.CO.O.CH_2R'$	Ser, Thr or HyLys with Asp/or Glu
Inter-chain ester with HyPro		HyPro with Asp or Glu
Oxazoline from inter-chain ester with hydroxylysine		HyLys with Asp or Glu
Inter-chain di-ester with carbohydrate		Carbohydrate with Asp and/or Glu

* The aspartyl and glutamyl residues are conceived of as being linked by means of their β- and γ-carboxyl groups, respectively.

removed at various times and added to 10 *N* hydrochloric acid, bringing them to *p*H 4 in order to stop the reaction. The samples were then dialysed exhaustively against distilled water to remove all free hydroxylamine. Simultaneously with the above reaction, a control mixture of gelatin, containing no hydroxylamine, was maintained at *p*H 10, sampled at intervals, and the aliquots dialysed as above.

The following determinations were then made: protein by a modification of the Kjeldahl method[6] and by the procedure of Lowry *et al.*[7]; N-terminal proline by the method of Piez *et al.*[8], in which terminal proline yields a diminished fraction of the colour given by free proline[9]; amino-groups by the ninhydrin method of Rosen[10]; and amide by the amount of alkali-distillable ammonia produced after hydrolysis with 1 *N* hydrochloric acid for 1 hr. Hydroxamides were measured by three independent spectrophotometric procedures: the classical determination of the magenta complex formed with iron salts such as ferric perchlorate; the quantitation of nitrite produced by oxidation with iodine[11]; and by a method developed in these laboratories, soon to be published, in which hydroxylamine, liberated from hydroxamides by hydrolysis with 1 *N* hydrochloric acid for 3 hr., reacts with indole to yield a coloured product. In addition to these analyses, the protein samples were diluted with equal parts of 1·8 per cent sodium chloride, and studied in a Spinco model *E* analytical ultracentrifuge at 59,780 r.p.m. at 40° C.

With all gelatins studied, the results show that alkaline hydroxylamine causes both a fast and a slow reaction, each characterized by the formation of protein-bound hydroxamide. The fast reaction, which is nearly complete in about 90 min. at 40° C. or in 3 hr. at room temperature, results in a decrease of molecular weight of the protein to a definite end-point of about 20,000. The sedimentation constant changes from the original value of about 3·3 S. for the α-component to a value of about 1·9 S. Fig. 1 shows sedimentation patterns for fish and calf parent gelatins before and after treatment with hydroxylamine, and clearly demonstrates the conversion of both the α- and β-components to the units of lesser molecular weight. During the course of the fast reaction there is no increase in free amino-groups to indicate the splitting of peptide bonds, nor can N-terminal proline be detected. Only late in the course of the fast reaction can one begin to measure a decrease in the number of amide groups; however, when the reaction is allowed to continue beyond this time, a gradual destruction of amide groups and even some cleavage of peptide bonds occur, characteristic of the second or slow phase of hydroxylamine activity.

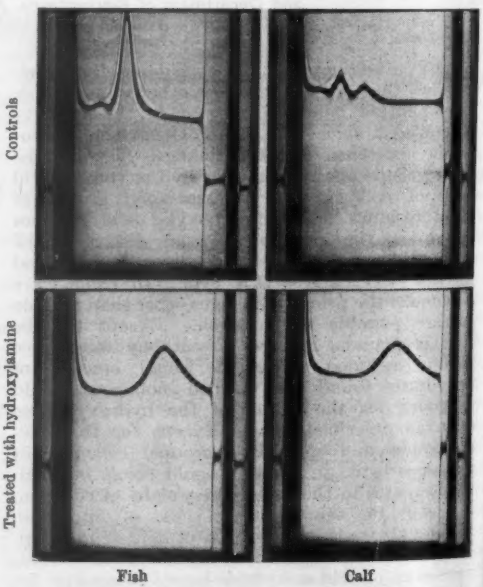


Fig. 1. The sedimentation patterns of parent gelatins obtained from calf and fish procollagens as influenced by treatment with hydroxylamine. The photographs were taken 100 min. after full rotor speed of 59,780 r.p.m. was attained

Only after about 24 hr. at 40° C., however, does treatment with hydroxylamine result in the formation of some dialysable material.

The protein-bound hydroxamide values determined by the methods previously described parallel one another and indicate that about 1 hydroxamide equivalent per 20,000 molecular weight unit is formed during the fast reaction with calf, fish, or acid-processed gelatin. The fast reaction with base-processed gelatin is characterized by the formation of about half this amount of bound hydroxamide, and the slow reaction with this gelatin also shows less hydroxamide production. Such a result is to be expected since ester-like bonds in gelatin could be disrupted by processing with base, and since gelatin produced in this manner contains about 60 per cent less amide nitrogen than does acid-processed gelatin.

We believe that the results summarized above establish the presence of ester-like or perhaps imide linkages between units or chains of about 20,000 molecular weight. It is these units which may be joined together in parent gelatin, or more specifically the α-unit of procollagen, which has a molecular weight of 70,000–80,000. It is of further interest that the components formed by treatment with hydroxylamine retain the capacity to form a gel when used in suitable concentrations of 3 per cent or more.

Grassmann *et al.*[12] and Konno and Altman[13] offered evidence for ester linkages in different collagens using the method of reduction with lithium borohydride. When finely pulverized acid-processed gelatin was suspended in anhydrous tetrahydrofuran containing lithium borohydride, and the mixture refluxed for 30 hr., we could not demonstrate changes in the ultracentrifuge pattern of the protein as compared with a suitable gelatin control; nor did the treated gelatin show a decrease in the amount of hydroxamide formed upon subsequent reaction with alkaline hydroxylamine. Thus it may be concluded that treatment of acid-processed gelatin with lithium borohydride under these conditions does not affect the internal ester-like bonds which are severed when the protein is made to react with hydroxylamine, although it may cause the reduction of terminal ester bonds in native collagens as described by the workers mentioned previously.

An inter-chain acyl shift appears unlikely as an explanation for the action of alkaline hydroxylamine, since the base favours the peptide form. However, in the case of an intra-chain ester-peptide acyl shift involving a residue of hydroxylysine, the ester form may be stabilized by the formation of an oxazoline ring, which, moreover, would probably be sensitive to treatment with hydroxylamine. Should this be the case, it may not be fortuitous that hydroxylysine is present in gelatin in an amount consistent with the bound hydroxamide formed after treatment with hydroxylamine, and this amino-acid might be serving a unique function of cross-linking.

We have begun experiments to separate the components of gelatin after treatment with hydroxylamine, and thus far have isolated two hydroxamide-positive fractions by electrophoresis on paper or starch at *p*H 4·0 in 0·05 *M* acetate buffer. It is our intention to degrade each of the components with bacterial collagenase, and, by suitable analysis, establish the nature of the hydroxylamine-sensitive linkages in gelatin.

Other proteins have been treated with alkaline hydroxylamine for 3 hr. at 40° C., and although several have yielded positive tests for protein-bound hydroxamide, none has formed as much as one-fifth the amount found when equivalent quantities of gelatin are used. Thus crystalline bovine serum albumin, insulin, salmine, and myosin gave detectable hydroxamide reactions, whereas ribonuclease and adrenocorticotrophic hormone did not. Except for albumin, ultra-centrifuge studies were not done, so that it cannot be ruled out that the bound hydroxamide was formed as a result of the splitting of terminal ester bonds or of particularly sensitive amide bonds. Treated albumin, however, showed no diminution of molecular weight as determined in the ultracentrifuge, but rather exhibited a peculiar, and as yet unexplained, aggregation of molecules.

It is also of interest that gelatin, esterified chemically, and afterwards treated with hydroxylamine, showed a large increase in protein-bound hydroxamide as compared with a suitable control.

Gelatins can also be treated with aqueous 1 *M* hydrazine at *p*H 8–9 at 40° C. for 2–5 hr. to cause a cleavage of the protein similar to, though less rapid than, that obtained with hydroxylamine. The products formed are less soluble than the corresponding hydroxamides, but, like the latter, retain a capacity for gelation. They are non-dialysable, and appear to be protein acid hydrazides which can react quantitatively with *p*-dimethylaminobenzaldehyde in acid solution, and which also can reduce copper salts quantitatively. The protein hydrazides can be treated with fluorodinitrobenzene to yield derivatives which are red under mild alkaline conditions (for example, producing red spots on filter paper when exposed to ammonia vapour), and which presumably are dinitrophenyl hydrazides. Both the protein hydrazides and the dinitrophenyl derivatives of these can be digested with collagenase, and the special chromatographic characteristics of the peptides formed are being used at present to establish the precise nature of the bonds cleaved by hydrazine.

In conclusion it may be stated: (1) that various kinds of gelatin are cleaved by either hydroxylamine or hydrazine under conditions favouring the disruption of ester bonds; (2) that these reactions occur without the concomitant splitting of peptide bonds; (3) that the products are non-dialysable proteins of about 20,000 molecular weight which react in one instance as hydroxamides and in the other as acid hydrazides. The results strongly suggest that ester-like bonds exist in parent gelatin, and that the specific disruption of these releases fundamental sub-units of the protein molecule.

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LETTERS TO THE EDITORS

PHYSICAL SCIENCES

Corpuscular Radiation and the Acceleration of Artificial Satellites

In a previous communication[1] I showed that the secular accelerations of Satellites 1958 β 2 and 1958 δ 1 varied nearly in unison with the 10·7-cm. radiation from the Sun. This correlation sheds no light on the nature of the radiation responsible for the variations of density of the upper atmosphere, since the 10·7-cm. radiation—the variations of which are remarkably similar to those of the sunspot numbers—can obviously be considered only as a general index of solar activity. To obtain further clues on this subject I reduced anew all the available observations of these two Satellites (about 2,500 for 1958 β 2 and 9,000 for 1958 δ 1) with the best elements at hand and obtained more accurate accelerations at twice the original resolution. The results are plotted in Fig. 1, together with the 10·7-cm. solar radiation curve[2]. As can be seen, the correlation with the solar radiation is remarkably good, even in details, for the high-flying, spherical *Vanguard* Satellite 1958 β 2, for which accurate, well-distributed Minitrack observations were available throughout. For 1958 δ 1 the observations are mostly optical, less accurate and more irregularly distributed, with occasional periods of near-invisibility; this fact, together with the elongated shape, may have contributed somewhat to the poorer correlation in its case.

The procedure for computing the accelerations was as follows. Residuals of individual nodal (for 1958 δ 1) or perigee (for 1958 β 2) passages from empirical equations were plotted and ordinates on the residual curves were read off at intervals of 25 revolutions; differences were taken from the resulting table and accelerations were computed from them. The residual curve for 1958 δ 1 is a succession of long, smooth waves of great amplitude which are mainly due to the 27-day fluctuations. At only two spots in the whole curve is the smooth succession of long waves interrupted by a transient, short-lived but unmistakable secondary oscillation. The dates of these disturbances are July 8–9 and September 4, 1958; they are exactly coincident with the only two great geomagnetic disturbances that occurred during the life-time of the Satellite. Both magnetic storms followed, at the usual 1-day interval, the appearance of a flare of importance 3 on the Sun.

Although the original accelerations had a resolution of 25 revolutions, it appeared possible to obtain significant accelerations with a resolution of 10 revolutions around the critical dates, and these—computed at intervals of 5 revolutions—are plotted in Fig. 2, together with the 3-hr. geomagnetic planetary indices K_p. It is quite evident that in both the July and the September events there was no immediate response at the time when the flare appeared, and that the atmospheric disturbance—an increase in density—proceeded nearly in synchronism with the magnetic disturbance. The increase in the acceleration amounted to 40 per cent in the July 8 event and to a little more than 30 per cent in the event of September 4. These are actually lower limits, since the limited resolution may have smoothed out the peaks (with a resolution of 25 revolutions the peaks are much reduced, as can be seen in Fig. 1, where the two disturbances are marked (1) and (2) respectively). Since the amplitude of the 27-day oscillations did not exceed 20 per cent for this Satellite (1958 δ 1), it appears that the corpuscular radiation capable of causing a first-rate geomagnetic disturbance has a greater, although more transient, effect on the atmospheric density at the 200-km. level than the ordinary

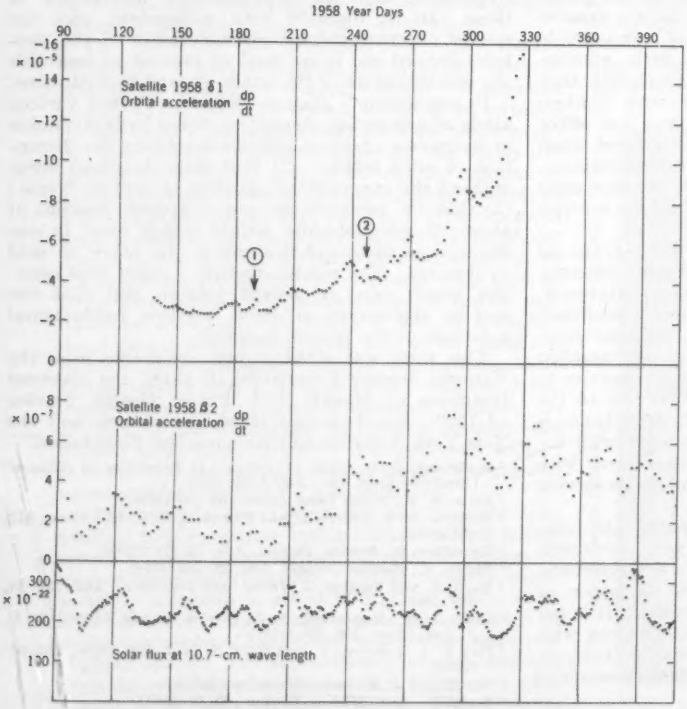

Fig. 1. Secular accelerations of Satellites 1958 δ 1 and 1958 β 2 compared with the 10·7-cm. solar flux. The dates of the two great geomagnetic disturbances, July 8–9 and September 4, 1958, are marked (1) and (2) respectively. Accelerations computed at intervals of 25 revolutions

fluctuation in solar radiation associated with the 27-day period. That this latter radiation is also corpuscular in nature appears quite probable, in view of the failure of the atmospheric density to react promptly to the stream of electromagnetic radiation which accompanies the appearance of a flare.

No disturbance could be detected in the residual curves for 1958 β 2 around the two critical dates in July and September. This may be due to the much smaller value of the acceleration for this Satellite, which would make a short-lived perturbation difficult to detect. It appears quite reasonable, however, that during a violent magnetic storm the atmospheric layers in the 200-km. zone are more strongly affected than those at a height of 600–700 km. If we may judge by the observed increase of the 27-day fluctuations with height[1], it appears that in ordinary conditions the opposite situation prevails, with the higher levels affected more than the lower.

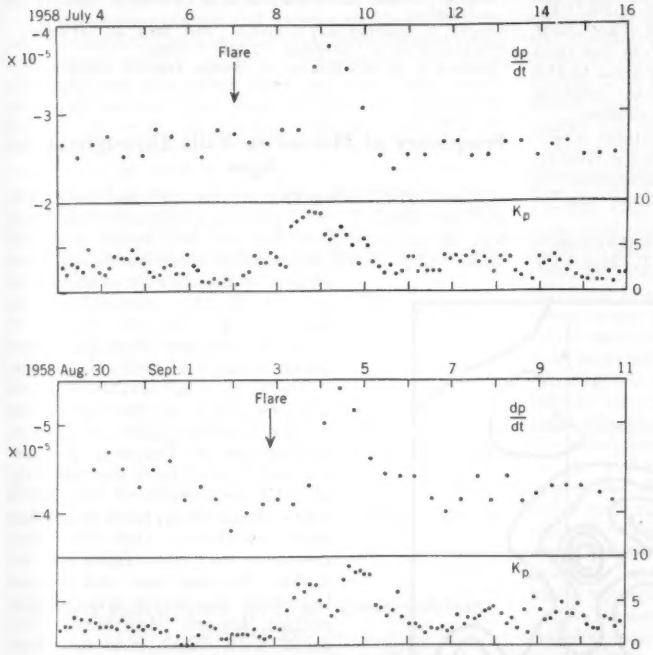

Fig. 2. Secular accelerations of Satellite 1958 δ1 computed with a resolution of 10 revolutions around the dates of two great geomagnetic disturbances and compared with the 3-hr. geomagnetic planetary indices K_p. The instants of the flares that preceded the magnetic storms are marked on the diagram

It might be of interest to remark that at the time of the July disturbance the latitude of perigee of 1958 δ1 was +35°; at the time of the September disturbance it was +15°.

I am indebted to Mr. Robert Briggs for most of the reductions of the *Vanguard* Satellite observations.

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A High-Resolution Survey of the Coma Cluster of Galaxies at 408 Mc./s.

In December 1958 an area of sky including the Coma cluster of galaxies was surveyed using the 250-ft. radio telescope at Jodrell Bank[1] with a Dicke-type radiometer, pass-band 408 ± 2 Mc./s., and noise figure 6 db. The beam was elliptical, having half-power widths of 40 and 56 minutes of arc. The system was calibrated using the radio source 14*N*5*A*, which was assumed to have a flux of 47×10^{-26} W. m.$^{-2}$ (c./s.)$^{-1}$ (ref. 2). The intensity contours for the region are shown in Fig. 1; Fig. 2 is a sample scan through the cluster and Table 1 gives the fluxes and positions of sources which can be resolved from the extremely complex background.

These results confirm the earlier report by Seeger, Westerhout and Conway[2] that radiation is detectable from the vicinity of the Coma cluster. The new feature of the present survey is the resolution of this radio emission into at least three distinct sources, *A*, *B* and *C* (see Table 1). Of these sources, *A* and *B* are point sources (that is, less than 30 minutes of arc in diameter) since they do not broaden the beam. Source *C* is appreciably extended and could consist of two sources separated by 40 minutes of arc. Although it is apparent from inspection of Fig. 1 that there is a concentration of sources near the centre of the cluster, it is possible that these are not associated with the cluster but are fortuitously coincident with it. The probability of such a coincidence is about 1 in 30. This figure is derived from the number-intensity diagrams given by Mills, Slee and Hill[4] in their survey of radio sources in the southern hemisphere. Thus it is reasonable to assume that some of these sources are physically associated with the cluster.

Current views are that the radio emission from clusters of galaxies may be separated into three components, namely, the normal emission from galaxies[5], the emission from intergalactic gas[6], and emission from abnormal systems. It is important to decide on the relative contributions from these three components for the Coma cluster. Baldwin and Elsmore[7] have shown that in the Perseus cluster

Table 1. FLUXES AND POSITIONS OF SOURCES

Source	R.A.	Dec. N.	Flux ($\times 10^{-26}$ W. m.$^{-2}$ (c./s.)$^{-1}$)	Notes
A	12h. 51m. 20s.	27° 57′	6	Probable error of position, ± 5′
B	12 51 20	26 39	2	,, ,,
*C**	12 56 00	28 24	12	,, ,,
D	13 08 10	27 48	6	,, ,,
E	13 16 30	30 10	5	,, ,,
F	12 44 00	26 13	3	Probable error of position, ± 10′
G	12 58 00	30 12	2	,, ,,
H	13 06 30	29 13	1	,, ,,
J	13 12 30	29 11	2	,, ,,
K	13 03 30	24 53	1	,, ,,
L	13 07 00	26 13	1	,, ,,

* Distributed source.

75 per cent of the emission at 80 Mc./s. originates in an abnormal object (*NGC* 1275), which is two galaxies in collision, and the remaining 25 per cent in a much broader component. We have measured the flux from *NGC* 1275 at 408 Mc./s. and find it to be 36×10^{-26} W. m.$^{-2}$ (c./s.)$^{-1}$. As the flux of the sources *A*, *B* and *C* is within an order of magnitude of this, they could be peculiar objects within the Coma cluster.

In the presence of these sources, which we shall provisionally assume to be associated with peculiar objects, it is difficult to determine whether a broad component of radio emission exists which is co-extensive with the optical limits of the cluster. If the general background brightness temperature is 2° K., the total emission from the cluster is $26 \pm 6 \times 10^{-26}$ W. m.$^{-2}$ (c./s.)$^{-1}$. The total emission from the sources in the cluster is $20 \pm 5 \times 10^{-26}$ W. m.$^{-2}$ (c./s.)$^{-1}$. Therefore if a distributed component exists it does not contribute more than 25 per cent to the total emission. This sets an upper limit to the possible contribution from the intergalactic medium and from all the normal galaxies in the cluster.

The outstanding problem is to determine which of the discrete sources are associated with the cluster. For this purpose accurate position measurements are required so that optical identifications can be made.

We thank Prof. A. C. B. Lovell for making available the facilities at Jodrell Bank, and Mr. R. Hanbury Brown for suggestions and criticisms throughout the course of the work. One of us (D. S. M.) gratefully acknowledges a Leverhulme Research Fellowship.

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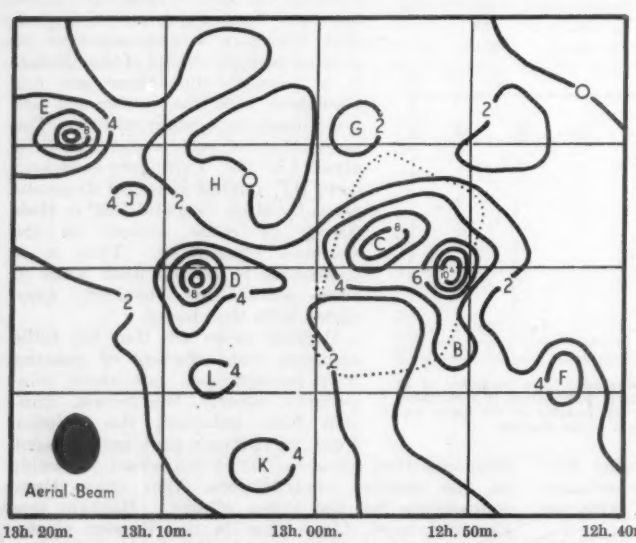

Fig. 1. 408 Mc./s. radio contours of the Coma cluster region (R.A. 12h. 55m., dec. + 28° 20′) plotted in intervals of 2° K. brightness temperature indicating the temperature above a chosen reference region (R.A. 12h. 40m., dec. + 28°). The dotted line is the equal population contour representing 400 galaxies per square degree (ref. 3). The co-ordinates are for epoch 1950.0

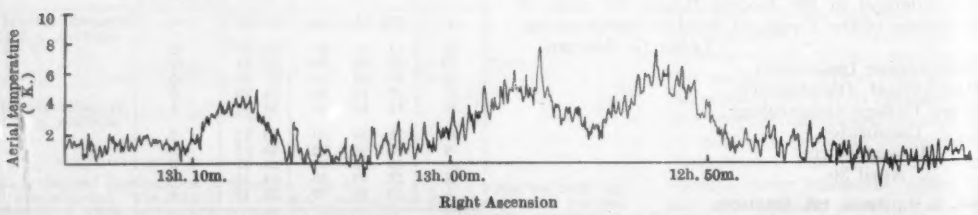

Fig. 2. A typical drift scan through the Coma cluster at declination + 28° 05′

Frequency of Meteorite Falls throughout the Ages

Prof. Hans Pettersson has pointed out[1] that the late Prof. F. A. Paneth's statement that "iron and stone meteorites did not fall before the later Quaternary"[2] must be modified since cosmic spherules formed by the ablation of meteorites in the Earth's atmosphere are found in the deepest sections of cores of sediments from the Pacific Ocean dating back to Tertiary times.

There is other evidence which suggests that meteorites have fallen on the Earth's surface at least as far back as the Tertiary. Henderson and Cooke[3] have described the unusual occurrence of the Sardis coarse octahedrite, from which they have concluded that this iron meteorite may have fallen into the middle Miocene sea and become buried by the slowly accumulating middle Miocene 'Hawthorne formation' with which it is now associated. Of even more importance is a previously unpublished report that an iron meteorite was found at a sub-surface depth of 1,525 ft. in an oil well drilled in Zapata County, Texas. The circumstances of the find have been described by M. Williams and forwarded in a personal letter dated September 14, 1956, by E. A. Wendlandt, chief geologist of the Humble Oil and Refining Company.

During the drilling of the well in 1930 an extremely hard object was struck at a depth of 1,525 ft. and in sediments of almost certainly Eocene age. This object was recovered and found to be a piece of metal about the size of a man's fist. An approximate analysis of a 0·4-gm. sample by M. Williams indicated about 82 per cent iron, 5–10 per cent nickel and traces of phosphorus, carbon and silicon. Two surfaces were polished and on etching Widmanstätten figures were quite distinctive. It would seem almost certain that the metal fragment was an octahedrite iron meteorite, but unfortunately the specimen has since been lost. There does not seem to be any reason to doubt the authenticity of the find, and the fact that the meteorite was embedded in Eocene sediments at a considerable depth from the surface could only mean that the meteorite fell in Eocene times.

Recent determinations of 'cosmic-ray ages' of certain meteorites indicate that they were broken from their parent bodies about 10^9 years ago[4]. In these circumstances meteorites have probably been available to fall on the Earth since at least early Palæozoic times. However, most meteorites are unstable on the Earth's strongly oxidizing surface, so that it would be most unlikely that they would be preserved in any readily recognizable form in sediments older than Tertiary. Since cosmic spherules are already largely fused and oxidized, they might be expected to be more stable and perhaps preserved in sediments considerably older than Tertiary.

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Some Experiments with an Electrodeless Discharge

Although various thermonuclear devices such as *Scylla*[1] employ electrodeless discharges to generate strong shock waves in a gas such as deuterium, with the exception of some exploratory experiments of Janes and Patrick[2], there are no reports of attempts to study the basic phenomena in such devices. An investigation of electromagnetically driven shock waves generated by this method is therefore being undertaken at the Atomic Weapons Research Establishment, Foulness.

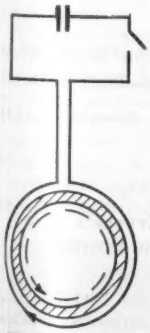

Fig. 1. Diagram of electrodeless apparatus for producing converging shock waves

The apparatus used is shown schematically in Fig. 1. A single-turn coil is wrapped around a 'Perspex' cylinder with 'Perspex' end-walls. The dimensions of the 'pill box' so formed were 3·1 cm. diameter by 2·2 cm. long, and this chamber was filled with deuterium to some known pressure between 0·1 and 2 mm. mercury.

A rapidly rising current was produced in the single-turn coil by suddenly switching an 8 μF. low-inductance condenser bank across it. Under certain conditions of gas pressure, and aided by some pre-ionization, the e.m.f.'s induced in the gas are sufficient to cause breakdown and a cylindrical current sheet is formed in the gas at the circumference of the pill box. This induced current is in the opposite sense to that in the coil, and the repulsion between the two current sheets gives rise to a strong shock wave in the gas.

Streak camera photographs (that is, radius–time curves) of the motion of the shock front showed that the shock did not reach the centre of the pill box. This result is in accordance with the observations of Janes and Patrick[2] and is attributed to some of the magnetic field from the current in the coil penetrating the discharge before the plasma formed by the shocked gas is sufficiently conducting to prevent diffusion of the magnetic field. This weak magnetic field is then trapped and amplified by the imploding shock until the magnetic pressure is sufficient to reduce the shock to rest.

This theory was confirmed by measurements of the magnetic field made with a coil mounted at the axis of the discharge; these showed a sudden rise as current began to flow in the coil, followed by a period of relatively constant field as the discharge built up and the gas became conducting. There was then an increase in magnetic field corresponding to the implosion of the shock wave. It was, moreover, found possible to make the shock travel much closer to the axis by putting on a d.c. field to cancel out the field trapped by the imploding shock. The degree of convergence is greatly increased by having a d.c. field of the order of 1,000 oersteds. For fields greater than this, the degree of convergence falls again, since the effect has been over-corrected. Also, with fields of the order of 1,000 oersteds, the speed of the imploding shock is increased, presumably because little work is done in compressing the small trapped magnetic field. This may well have important applications to the production of high-temperature plasmas, since the temperature of a shocked gas varies approximately as the square of the shock speed.

The interaction of the imploding shock with the trapped magnetic field is of some interest since it is an addition to the small number of cases where hydromagnetic interactions may be observed on the laboratory scale with magnetic Reynolds numbers in excess of unity. The magnetic Reynolds number may be defined by:

$$R_m = 4\pi\sigma U r_0 \qquad (1)$$

where σ is the conductivity of the plasma (e.m.u.), U is the shock speed (cm./sec.) and r_0 is the radius of the apparatus (cm.).

The physical significance of magnetic Reynolds number is that, in hydromagnetic phenomena, the magnetic lines of force diffuse through the medium or are fixed to conducting particles of the medium if R_m is much less or greater than unity respectively. On the cosmic scale, the linear dimensions r_0 are so large that R_m is normally much greater than unity. On the laboratory scale, however, r_0 is usually limited to the order of ten centimetres, and most attempts to study hydromagnetic phenomena at large R_m have employed highly conducting liquids. The present experiment achieves a high R_m by means of high velocity, U, and fairly high conductivity, σ.

A theory of the variation of trapped magnetic field with shock radius has been formulated and the results presented as a power series in $1/R_m$. The first two terms give a field variation in the form:

$$H \propto \{r(r + \delta)\}^{-(1+1/\eta)} \qquad (2)$$

where $\delta = \eta/2\pi\sigma U$, and r is the shock radius (cm.) at time t (sec.), and η is the ratio of densities across the shock front.

This variation has been computed for various shock speeds, the temperature of the shocked gas (and hence σ, ref. 3) being determined from the Rankine – Hugoniot equations taking the ionization and dissociation energies of deuterium into account. The results are presented in Table 1. It will be seen how rapidly the magnetic Reynolds number increases and the distance δ decreases with increasing shock speed.

Table 1

Shock speed (cm./μsec.)	Temperature of shocked gas (° K.)	Conductivity σ (E.M.U.)	Magnetic Reynolds number for apparatus 1-cm. radius	Compression (η)	δ (cm.)
6·0	$5{\cdot}0\times10^4$	$2{\cdot}8\times10^{-7}$	2·1	7·8	0·74
7·0	$7{\cdot}9\times10^4$	$5{\cdot}7\times10^{-7}$	5·0	6·3	0·25
8·0	11×10^4	$9{\cdot}3\times10^{-7}$	9·3	5·6	0·12
9·0	15×10^4	15×10^{-7}	17	5·3	0·06
10·0	19×10^4	21×10^{-7}	26	5·0	0·04

Equation (2) may be expressed in the form:

$$\frac{1}{H}\frac{dH}{dt} = -U\left(1 - \frac{1}{\eta}\right)\left\{\frac{1}{r} + \frac{1}{r+\delta}\right\} \qquad (3)$$

The values of $\frac{1}{H}\frac{dH}{dt}$ obtained theoretically from this formula have been compared with the values obtained experimentally with the small field-measuring coil. Preliminary measurements indicate that the theory accounts reasonably well for the experimental results.

Another phenomenon that has been observed is that the gas will not break down on the first half-cycle of the oscillatory current in the single-turn coil when the condenser bank is switched across it if the ambient deuterium pressure is below a certain critical value.

An approximate theory of this effect has been derived by postulating that electrons multiply by a Townsend ionization-by-collision mechanism until a certain cut-off time. Providing this time is longer than a certain amount, the gas is sufficiently ionized to become highly conducting and then breaks down with the formation of a shock wave on the first half-cycle. If it is supposed that the cut-off time is determined by a spiralling motion of the electrons in the rising magnetic field due to the current flowing in the single-turn coil, and that when the field has risen above a certain amount the electrons do not travel a sufficient distance in the direction of the induced electric field to have enough energy to ionize by collision, it can be shown that a shock wave is formed on the first half-cycle only if:

$$r_0 V\alpha \gtrsim 6{\cdot}5 \times 10^4 \qquad (4)$$

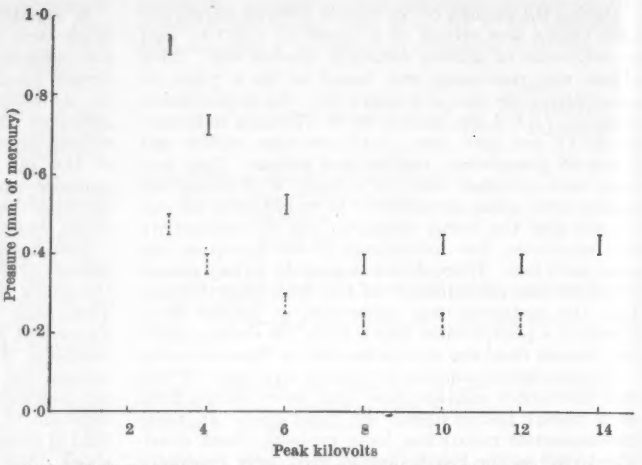

Fig. 2. Minimum breakdown pressure at various voltages for electrodeless discharges of 3·1-cm. diameter (full lines) and 6·2-cm. diameter (broken lines)

where r_0 is the radius of the discharge chamber (cm.), V is the voltage across the coil, α is Townsend's first ionization coefficient.

α is a function of the applied electric field (X in V./cm.) and gas pressure p (in mm.). Experimentally determined values of the minimum pressure at which the first quarter-cycle of the primary voltage produces breakdown are shown in Fig. 2. These values are for two geometrically similar sets of apparatus of different radii. The dependence of the minimum pressure for breakdown on voltage at a given radius is approximately in accord with equation 4, while the predicted dependence on radius for a given voltage is almost exactly fulfilled.

Values of α/p calculated from these curves by means of equation 4 are about twice as large as those determined by Hale[4] and Rose[5]; but in view of the approximations made in the theory, this is considered to be satisfactory agreement.

A full account of the experimental and theoretical work will be published elsewhere. We are indebted to the Director of the Atomic Weapons Research Establishment for permission to publish this communication.

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Atomic Weapons Research Establishment,
Foulness, Southend-on-Sea,
Essex. May 7.

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Use of Synthetic Quartz Crystals as a Support for Specimens in Powder Photographs

In the course of experiments on the hydrothermal growth of quartz crystals from aqueous alkaline solution by the temperature gradient method[1], thin needle-shaped crystals were obtained from the autoclave adhering to the cooler portions of the inside

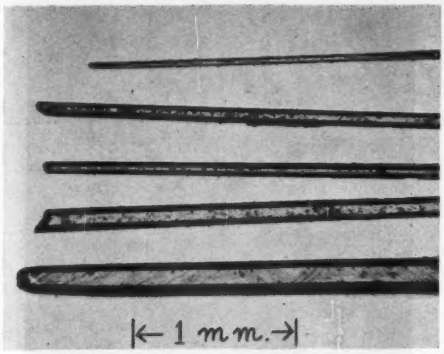

Fig. 1. Examples of needle-shaped synthetic quartz crystals of varying diameters

wall, to the bottom of the lid, and to the stainless steel support for crystal seeds. The autoclave was made of chromium steel, set vertically and heated from below. The solution contained 2 per cent sodium hydroxide and 3 per cent sodium carbonate by weight. The approximate temperature and pressure of the autoclave were 350–360° C. and 300 atm., respectively. The needles were obtained in large numbers pointing almost at random. The diameters of individual needles were uniform and ranged from 0·03 to 0·2 mm. Their lengths were 5 mm. or more. Typical examples are shown in Fig. 1.

The crystals were elongated in the *c*-axis direction with side surfaces bounded by six prism faces and capped at the free end by three uncompleted major rhombohedral faces. Parallel striations usually observed on the prism faces of natural quartz crystal were not observed with the present ones. Each needle was uniform in cross-section and straight in the *c*-axis direction.

The crystal needles serve as excellent supports for powder specimens in the X-ray Debye–Scherrer technique, since they are strong, perfectly stable to air and to X-irradiation, and highly resistant to heat and chemicals. They can be used repeatedly. Powder samples were fixed to the needle with a thin layer of grease. The adjustment making the long axis of the needle coincident with the rotation axis of the specimen holder of the camera could be carried out accurately, since a sufficient length of supporting crystal was straight. The centring of the powder specimen was performed automatically with this adjustment.

Debye–Scherrer photographs taken by this method are shown in Fig. 2. There is no confusion between the patterns of the quartz and the powder, the former appearing as short specks superimposed on the usual Debye–Scherrer pattern. The diameter of the camera was 57·3 mm. The collimator consisted of two pinholes 0·3 mm. in diameter and 51·5 mm. apart. The camera was equipped with a small goniometer head as a specimen mount.

Fig. 2. X-ray powder pattern of germanium and germanium dioxide mixture taken with needle-like synthetic quartz crystal as the specimen support. Radiation, copper $K\alpha$ (filtered)

This method has the following advantages.

(1) The photographs are free from diffuse halos, unavoidable when glass fibres or other amorphous materials are used. This increases the sensitivity for the detection of weak lines.

(2) The equatorial line is easily determined from the rotating crystal pattern of quartz. The centres, $2\theta = 0°$ and 180°, are easily and accurately obtained.

(3) It gives valuable information on the X-ray spectra giving rise to the powder pattern. Copper $K\beta$, tungsten $L\alpha_1$, or other spectra due to impurities in the target can be recognized by observation of the diffraction spots due to the quartz crystal. The relative intensities of the spectra are also easily obtained.

(4) Departures of the specimen from the correct orientation during exposure is easily detected; the rotation photographs of quartz become similar to 'tilted-crystal' photographs, the diffraction spots becoming longer with the increase in 2θ.

(5) The quartz pattern serves as a good standard for interpolation in the measurement of spacings of the powder pattern. Recently, the variability of lattice constants of quartz crystal by amounts as large as 0·01 per cent has been reported by Keith[2]. Her result shows that the lattice constant, a, of synthetic quartz varies from 4·9126 to 4·9131 A., depending on the conditions of its growth. However, for measurements where this amount of variation is negligible synthetic quartz may well serve as a standard.

Measurement of the precise lattice constants of our needle crystals and their variation, if any, under X-irradiation are in progress.

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March 16.

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METALLURGY

Crystallographic Orientation of Nodules formed by the Grain Boundary Reaction of Aluminium–Zinc Alloys

Although the grain boundary reaction phenomenon (discontinuous precipitation or cellular precipitation) of aluminium–zinc alloys was reported previously[1,2], the crystallographic orientations of nodules have not yet been determined. As the spacing between lamellar precipitates in nodules of aluminium–zinc alloys is very small, the precipitates cannot be observed by the ordinary optical microscope, but only by the electron microscope, as shown in Fig. 1. If the precipitates in the nodules are oriented in a definite direction, the relationship of the crystallographic orientations between nodules and matrix grains could be determined[3]. Or, if the specimens can be treated by an adequate etching technique to be sensitive to polarized light, the relationship of the orientations can be observed under the polarized illumination[4]; but this was also impossible for the existence of the small precipitates.

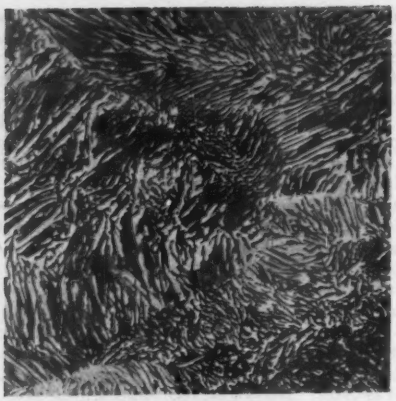

Fig. 1. 25 per cent zinc aged for 250 hr. at 120° C. (× 3,000)

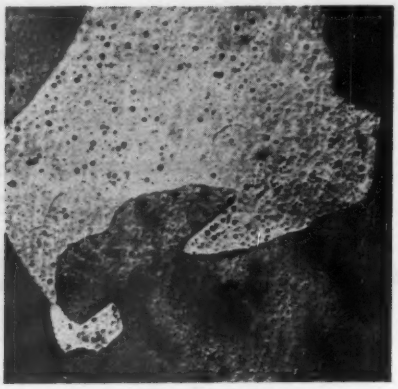

Fig. 3. The same as Fig. 2 except for further heat treatments for 5 sec. at 470° C. (× 40)

Fig. 2. 25 per cent zinc aged for 600 hr. at 50° C. (× 40)

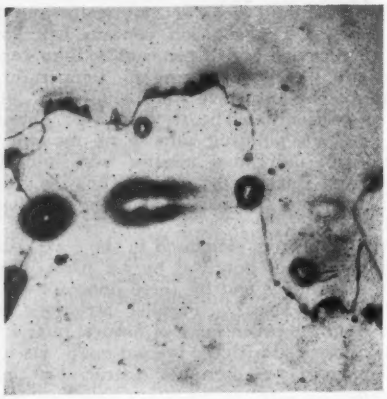

Fig. 4. 25 per cent zinc aged for 10 hr. at 100° C., heat treated for 5 sec. at 470° C. and re-aged for 1½ hr. at 100° C. (× 200)

However, we were able successfully to determine the crystallographic relation by the optical microscope under the polarized illumination after adequate heat treatment and chemical polishing. The technique is as follows. The specimens with nodules produced by ageing, as shown in Fig. 2, are solution heat-treated for a short time, for example about 5 sec., in a salt bath regulated at 470° C. Thus the bulk of precipitates in nodules are dissolved into the matrix and the advancing boundaries of nodules are left as they were. This fact can be observed by means of an optical microscope under polarized illumination after chemical polishing in a solution of 25 parts of sulphuric acid, 70 parts of ortho-phosphoric acid and 5 parts of nitric acid at 135° C., as shown in Fig. 3. In this case, it is most important to keep the temperature of solution at 135° C. exactly, otherwise the specimens are not sensitive to polarized light. The result, clearly shown in Fig. 3, is the same as those obtained in $AlAg_3$, $ZnAg_4$ and $ZnCu_5$ alloys; that is, the crystallographic orientation of a nodule is the same as that of the adjoining grain from which the nodule grew. Moreover, when specimens so treated are aged again, some new nodules are formed at those parts of the advancing boundary which is parallel to the initial grain boundary, but not at the boundary which is normal to the initial one (Fig. 4). This indicates that the growth-rate of nodules depends on the relative orientation of the grain boundaries[6].

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CHEMISTRY

Alicyclic Structures in Coals

X-ray and other physical and chemical evidence indicates that coals assume a graphitic structure with metamorphism. X-ray diffraction patterns of some meta-anthracites show (*hkl*) reflexions that arise from the three-dimensional unit cell of a graphite lattice[1,2]. For the anthracites, a number of diffraction bands

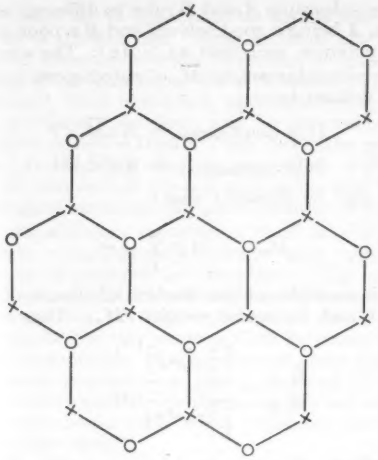

Fig. 1. The molecule used in the calculations. (For the alicyclic case carbon atoms shown as crosses were taken to be in one plane, those as circles in another, that is, a chair configuration)

can be detected which coincide with the (*hk*) reflexions of aromatic layers (having a two-dimensional unit cell of side approximately equal to that of graphite layers)[3]; the remainder of the bands can be attributed to the (00*l*) reflexions of these layers packed 'turbostratically'[4]. Medium- and low-rank coals also give diffuse diffraction patterns that can be indexed as the (*hk*) and (00*l*) reflexions of aromatic layers. These observations led to the development of methods for quantitative analysis of X-ray intensity profiles of coals, permitting estimation of bond-lengths, fraction of carbon in aromatic layers (the remainder assumed to be roentgenamorphous), and size distribution and stacking of the layers[3,5,6].

Coal components rich in hydrogen (more than 6 per cent by weight), for example, exinites[7], resins and some coalified wood[8], show diffraction bands in the angular region where the (*hk*) reflexions of aromatic layers occur. However, the patterns of these components do not have the so-called (002) band. Instead they have a very pronounced peak at smaller angles corresponding to *d*-spacings ranging from 4·3 to 5 A. Despite this ambiguity, exinites were still considered as consisting mainly of condensed aromatic layers, the shift in *d*-spacing being attributed to buckling of the layers or to the presence of more aliphatic material around the edges of the aromatic systems[7].

Treatment of vitrinites with lithium–ethylenediamine results in the addition of considerable hydrogen (as many as 44 hydrogen atoms/100 carbon atoms)[9]. X-ray patterns of treated vitrinites showed extensive changes at moderately low scattering angles[10]. The (002) peak was displaced to lower angles, corresponding to an increase in *d*-spacing from about 3·6 to 4·6 A. Since lithium–ethylenediamine was found to be a powerful hydrogenating agent for aromatic rings without cleavage of C—C bonds[11], the shift of the (002) band to larger *d*-spacings appeared to be consistent with conversion of planar aromatic layers to buckled hydrogenated rings. However, intensity patterns of medium- and high-rank coals treated with lithium–ethylenediamine showed only small changes at high angles, and this seemed incompatible with conversion of aromatic structures to alicyclic or hydroaromatic (partially reduced) structures[9]. Therefore theoretical work on the scattering of alicyclic molecules appeared desirable. Preliminary results of this analysis are presented here, and full details will be published later.

An aromatic molecule containing 30 carbon atoms (Fig. 1) has been chosen as an example. The intensity $J(s)$ in atomic units, scattered at any angle 2θ, was computed by the Debye formula[12]:

$$J(s) = \sum_r \frac{n(r)}{N} \frac{\sin 2\pi rs}{2\pi rs}$$

where $s = 2 \sin \theta/\lambda$, $n(r)$ is the number of interatomic distances of length r within the layer, irrespective of direction, and N is the total number of atoms in the layer. All carbon bonds were taken to be of equal length, 1·4174 A. (cf. ref. 5), and all inter-bond angles to be 120°. After constructing the Patterson function[13] of the molecule and inverting the result by the Debye formula, intensity data were computed numerically on the *Univac* 120. The results are shown in Fig. 2 (dotted line).

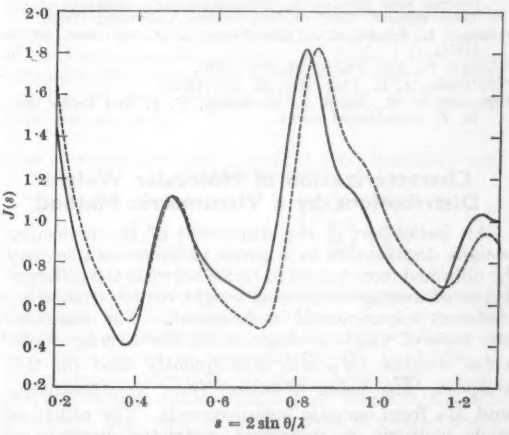

Fig. 2. Calculated X-ray scattering intensities for an aromatic (dotted line) and an alicyclic molecule (solid line) containing 30 carbon atoms (cf. Fig. 1)

A configuration of the alicyclic counterpart of the molecule shown in Fig. 1 was next considered. Carbon atoms shown in the figure as crosses were taken to be in one plane and those as circles in another. All carbon bonds were taken to be 1·5420 A. in length and all inter-bond angles 109° 28·4′, the tetrahedral bond angle. The computed curve is shown in Fig. 2 (solid line).

It is seen that alicyclic molecules give rise to diffraction bands at approximately the same angular region where the (*hk*) reflexions of aromatic molecules occur. The peaks employed for quantitative profile analysis are the (10) and (11) bands of aromatic layers, the first and second peaks from the left, respectively. For the alicyclic molecule shown, the second peak is displaced to a lower angle; however, for other isomeric configurations the displacement is less or even non-existent[14]. It appears that alicyclic or hydroaromatic structure may account to a large extent for the intensity patterns of coal components rich in hydrogen. Indeed, low-rank and even medium-rank coals may contain alicyclic structures in appreciable amounts; diffraction bands of these coals at high

angles can be explained by the presence of both alicyclic and aromatic structures. Consequently, it appears that previous quantitative analyses of the X-ray patterns of coal should be re-examined. We wish to thank R. W. Smith for the *Univac* computation.

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Characterization of Molecular Weight Distributions by a Viscometric Method

An indication of the dispersion of the molecular weight distribution in a given polymer sample may be obtained from values of ratios between the different types of average molecular weight as determined by different experimental techniques[1]. For example, the ratio of weight-average to number-average molecular weights ($\overline{M}_w/\overline{M}_n$) is frequently used for this purpose, $\overline{M}_w$ being obtained from light-scattering and $\overline{M}_n$ from osmotic measurements. The utility of such methods is somewhat restricted because of the uncertainties associated with determinations of the individual molecular weight averages in certain regions of the molecular weight range. Osmometry, in particular, is less accurate for both low and very high molecular weights. Since the percentage error in the derived ratio is approximately the sum of percentage errors of the individual determinations, it is evident that quoted values of $\overline{M}_w/\overline{M}_n$ in the region of 1·05 (typical of a sharp polymer fraction) must be accepted with reserve, because such a figure implies a greater accuracy in the individual methods than their current state of development would appear to warrant. If similar ratios could be obtained by the use of a single technique part of the uncertainty as to the magnitude of possible error would be eliminated. It is the purpose of this communication to propose a method for characterizing the dispersion of a molecular weight distribution based on the measurement of limiting viscosity numbers alone.

The method requires a knowledge of the constants K,a of the Mark–Houwink equation relating the limiting viscosity number $[\eta]$ to the molecular weight M of monodisperse polymer:

$$[\eta]_A = K_A M^{a_A} \quad (1)$$

$$[\eta]_B = K_B M^{a_B} \quad (2)$$

Here the subscripts A and B refer to different solvent systems, A being a good solvent and B a poor solvent for the polymer, such that $a_A > a_B$[1]. The viscosity-average molecular weight $\overline{M}_v$ of polydisperse polymer is then defined by:

$$[\eta]_A \text{ (polydisperse)} = K_A \overline{M}_{vA}^{a_A} \quad (3)$$

$$[\eta]_B \text{ (polydisperse)} = K_B \overline{M}_{vB}^{a_B} \quad (4)$$

It can also be shown[1,2] that:

$$\overline{M}_v^a = M_0^a \sum_1^\infty w_r r^a \quad (5)$$

in which w_r is the weight fraction of species of chain length r and molecular weight rM_0. Thus:

$$\frac{\overline{M}_{vA}}{\overline{M}_{vB}} = \frac{\left[\sum_1^\infty w_r r^{a_A}\right]^{1/a_A}}{\left[\sum_1^\infty w_r r^{a_B}\right]^{1/a_B}} \quad (6)$$

from which it may be shown, using Hölder's inequality, that:

$$\overline{M}_{vA} > \overline{M}_{vB}, \text{ if } a_A > a_B \quad (7)$$

It follows that the ratio $\overline{M}_{vA}/\overline{M}_{vB}$, which is a function only of a_A, a_B and the molecular weight distribution of the polymer, may serve as an indication of the degree of polydispersity.

In order to illustrate the application of the method to particular cases it is convenient to use the generalized exponential distribution[3,4]:

$$w_r \, dr = \frac{r^z \lambda^{z+1} \exp(-\lambda r)}{\Gamma(z+1)} \, dr \quad (8)$$

where

$$\lambda = z/\bar{r} = (z+1)/\bar{r}_w \quad (9)$$

and in which $\bar{r}$, $\bar{r}_w$ are the number- and weight-average chain-lengths respectively. z is a number which characterizes the type of distribution. Thus $z = 1$ corresponds to the so-called 'most probable' distribution, $z = 2$ to the distribution for low-conversion vinyl polymerizations in which termination occurs exclusively by combination of radicals[3], and increasingly larger values of z correspond to increasingly sharper distributions such as may be expected for fractionated polymer. For this generalized distribution it can be easily shown that:

$$\frac{\overline{M}_{vA}}{\overline{M}_v} = \left[\frac{\Gamma(z+1+a_A)}{\Gamma(z+1)}\right]^{1/a_A} \left[\frac{\Gamma(z+1)}{\Gamma(z+1+a_B)}\right]^{1/a_B} \quad (10)$$

Values of this ratio are given in Table 1, together with the corresponding ratios, $\bar{r}_w/\bar{r}$, for various values of z, and for values of a_A of 0·80, 0·70, 0·60, assuming a_B to be equal to 0·50 (as in a theta-solvent).

The differences between the ratios $\overline{M}_{vA}/\overline{M}_{vB}$ for different distributions, although small, are suffi-

Table 1

z	$\bar{r}_w/\bar{r}$	$\overline{M}_{vA}/\overline{M}_{vB}$		
		$a_A = 0·80$, $a_B = 0·50$	$a_A = 0·70$, $a_B = 0·50$	$a_A = 0·60$, $a_B = 0·50$
1	2	1·080	1·053	1·027
2	1·5	1·052	1·035	1·017
5	1·2	1·026	1·017	1·009
10	1·1	1·014	1·009	1·005
20	1·05	1·007	1·005	1·002

ciently large to be detectable by simple viscometric methods in current use. These methods are capable of giving viscosity-average molecular weights to an accuracy of at least 1 per cent. Moreover, Hermans and Kooy[6] have described apparatus with which limiting viscosity numbers may be determined with a precision of better than 0·1 per cent. The proposed method is therefore directly applicable within the limits of precision at present attainable, and has the advantage of requiring only one technique of measurement. In addition, measurements of $\overline{M}_{vA}/\overline{M}_{vB}$ may be made comparatively quickly and only small quantities of polymer are necessary. The accuracy of the method will be affected less by errors in the absolute values of the constants of the Houwink equations for the two solvents than by errors in their relative magnitudes. Since values of these constants in different solvents are often obtained by viscometric determinations on the same polymer samples, little error should arise when constants derived in this way are used.

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Feb. 25.

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Direct Examination of the Degradation of High Polymers by Gas Chromatography

Examination of the degradation products of high polymers may give an indication of the mechanism of breakdown, and may also yield information about the structure and composition of polymers and copolymers. Such an examination may be quickly and conveniently effected if the degradation products are characterized by gas chromatography as they are formed. This may be achieved by generating a pulse of degradation products in the chromatographic apparatus, which is so designed that the products are swept into the column with the minimum delay and disturbance.

Two degradation techniques have been applied in this way. In the first method, a few milligrams of polymer sample are used as a dielectric which is broken down by triggering through it a high-energy discharge (up to 2,000 joules). This method has been applied to polyethylene, polyvinyl alcohol, polyvinyl acetate, polyvinyl chloride, polymethyl methacrylate, polystyrene and cellulose acetate. In all cases the degradation is very drastic, the principal products being small unsaturated molecules with acetylene in largest yield. In some cases (for example, polystyrene), 'characteristic' fragments (for example, toluene, styrene, α-methyl styrene) are also obtained; but the yield of these in comparison with the small unsaturated molecules cannot be significantly increased by variation of the experimental parameters. Hence this method is of only limited utility for structure determination, and the secondary thermal effects are so predominant that no information about the mechanism of the primary electrical breakdown is derived.

The second technique may be controlled to give any desired degree of thermal degradation, and has proved to be more versatile in application. The method involves coating or placing the polymer sample on a filament, which can quickly be brought to any specified temperature for a brief period. The temperature calibration of the filament is effected by optical pyrometry and also by melting-point observations. Samples up to several milligrams have been examined, and the sensitivity of the gas chromatography apparatus is such that less than 1 μgm. of a component in the degradation products may be detected.

Two principal applications of this second degradative technique are under investigation. The first is in the rapid estimation of copolymer composition. The procedure here is simply to degrade the copolymer sample at a particular temperature and to measure peaks on the chromatogram characteristic of each constituent of the copolymer; for example, for vinyl chloride – vinyl acetate copolymers the hydrogen chloride and acetic acid peaks have been used. Separate experiments on weighed samples of the pure homopolymers have yielded direct relations between their masses and the sizes of the characteristic peaks, and these relations enable the sizes of the peaks in the copolymer chromatogram to be translated into masses of the two monomers present. In Table 1 the results obtained for three vinyl chloride – vinyl acetate copolymers are compared with those obtained by infra-red analysis.

Table 1. Percentage Vinyl Acetate in Vinyl Chloride – Vinyl Acetate Copolymers

By infra-red analysis	By degradation
26·7 ± 1·0	26·2 ± 0·8
34·6 ± 1·0	31·4 ± 0·7
44·3 ± 1·0	43·9 ± 0·5

The second application of the technique consists of recording the degradation products from the same sample of polymer maintained for a few seconds at each of a series of temperatures up to 1,000° C. Polystyrene, polyvinyl chloride, polyvinyl acetate and polymethyl methacrylate have been examined in this way, and each polymer gives a characteristic 'spectrum'. (Under the conditions used, the whole temperature-range can be covered in less than half an hour, and the results give a rapid identification of unknown polymers.) The relative proportions of the degradation products at each temperature yield information about the relative importance of the various degradation processes throughout the temperature-range. For example, it can be shown in the case of polyvinyl acetate that simple depolymerization is a relatively insignificant process; that loss of acetic acid is the principal degradation process and occurs at intermediate temperatures, and that the resulting polyacetylene skeleton is much more stable and is degraded principally at the highest temperatures.

This procedure is also being applied to copolymer samples, and a method of distinguishing block from random copolymers is projected.

Full details of this work and of the modifications in hand will shortly be published elsewhere.

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Bond Energies of Some Alkali Fluorides

The attenuation of microwaves by flames containing traces of alkali metals may be used to determine the electron concentration, arising from the ionization of the alkali, in these flames[1,2]. If a halogen is added to such a flame, the electron concentration is reduced directly by the formation of negative ions, and indirectly by the formation of the alkali halide[3,4]. Flame photometric studies show that the halogens also reduce the excess hydrogen atom concentration in the flame[5]; but it has been observed that fluorine has the least effect[6]. This reduction makes it difficult to estimate the bond energies of the alkali halides from microwave studies[7], because of the indirect nature of the arguments used in the estimations[8]. In the case of fluorine, however, the argument is simplified by the unimportance of the negative ion effect, since the electron affinity of fluorine is low relative to the bond energies of the alkali fluorides, and by the much smaller disturbance produced in the hydrogen atom concentration. In addition, the observations are made at high temperatures where the hydrogen atom concentration approaches equilibrium[5] and it is possible to obtain reliable and consistent results, which it is of interest to place on record.

Sodium fluoride has been examined over the temperature-range 2,300–2,600° K., using perfluoromethyl*cyclo*hexane as source of fluorine, and the dissociation constant determined, assuming the dissociation energy of hydrogen fluoride[9] to be 134 kcal. A graphical determination of the dissociation energy (Fig. 1) gives a value of 124 kcal., equivalent to 119 kcal. at 0° K., in agreement with a statistical 'Third law' value of 120 kcal., calculated from the measurement at 2,400° K. It is also possible to estimate the difference between the bond energies of hydrogen fluoride and sodium fluoride directly from the experimental results. This difference is 14 kcal., which indicates that the bond energy of sodium fluoride is 120 kcal. This concordance shows that the disturbing effects described above are, indeed, negligible. Potassium fluoride could not conveniently be examined over this temperature-range; but a statistical calculation at 2,400° K. gave the bond energy as 117 kcal. Lithium fluoride could only be examined at the highest temperature (2,600° K.) and a statistical calculation put the bond energy at 141 kcal. These values are in good agreement with the thermodynamic predictions of Smith and Sugden[10] but differ somewhat from other authorities[11,12]. They are, however, in good agreement with some values determined by flame photometry in these laboratories recently[13].

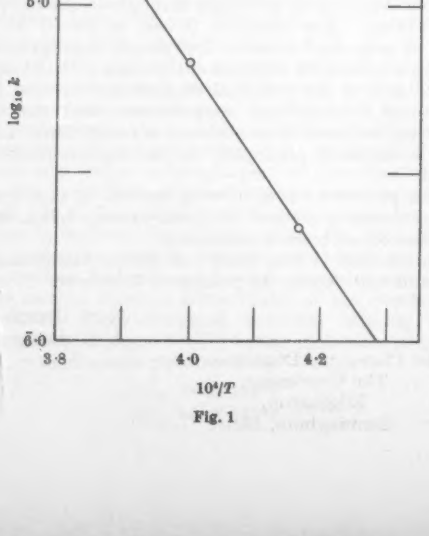

Fig. 1

Thanks are due to Imperial Chemical Industries, Ltd., General Chemicals Division, for the gift of chemicals.

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Tetrahedral Co-ordination in Ni(II)

Lingafelter[1] has suggested that the occurrence of blue-coloured Ni $en_2(NCS)_2$ and Ni $en(H_2O)_4(NO_3)_2$ for which X-ray evidence gives 6 co-ordination for the nickel atom contradicts the criteria for identifying tetrahedrally co-ordinated Ni(II) suggested in my earlier communication of the same title[2], with the implication that these criteria are invalid.

The basic criterion laid down[2] was the disappearance of the characteristic peak in the region of 400 mμ of octahedrally co-ordinated Ni(II), which usually has a green colour. With the disappearance of this peak, a deep blue colour would be expected to result, other things being equal. A property which would suggest the possibility of a tetrahedral 4 co-ordinate condition would therefore be possession of a blue colour. However, since a shift of the 400-mμ peak to slightly lower wave-lengths could give the same effect so far as the eye alone is concerned, verification by spectral analysis would be an obvious requirement.

Ballhausen[3] has used the absorption spectra of the various stages of complexing of Ni(II) with ethylene diamine to show the spectra of $[Ni\ en(H_2O)_4]^{++}$ and $[Ni\ en_3(H_2O)_2]^{++}$, and in fact the 400 mμ peak (as well as the others) has been shifted towards lower wave-lengths, but increased, not diminished, in intensity. Dr. R. G. Wilkins, of the University of Sheffield, has kindly shown me a solid preparation of the salt, $[Ni\ en(H_2O)_4](NO_3)_2$, which is light blue, and the absorption spectra he has obtained for ice-cold aqueous solutions of the salt correspond closely to that given by Ballhausen[3]. This blue-coloured complex cation therefore meets the criteria for 6 co-ordination of Ni(II), and it is no surprise that X-ray investigation of the related salts verifies this.

My first statement of the mode of identification[3] therefore still stands.

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It would appear quite possible that the disappearance of the 400-mμ peak is indicative of tetrahedral co-ordination of nickel(II), and I have no argument with this criterion. My communication was prompted by Katzin's apparent conclusion concerning the configuration of the blue compounds studied by Lifshitz, Bos and Dijkema on the basis of visual colour alone.

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A Colour Reaction for Thiosulphinates

Investigations of the chemistry of the thiolsulphinates, both synthetic and of natural provenance, would be aided if a rapid colorimetric test for these compounds were available. Aliphatic thiolsulphinates can be characterized, in part at least, by reaction with thiamine[1] or with cysteine[2] to yield crystalline derivatives; but these methods do not generally distinguish thiolsulphinates from thiolsulphonates, since the latter compounds will also react with these reagents. The addition of sodium nitroprusside[3] solution followed by base will yield orange to red colours in the presence of aliphatic thiolsulphinates or thiolsulphonates. This procedure has found application in paper chromatography[3] in investigations of the formation of thiolsulphinates in plants of the genus *Allium*. In our experience, the colour, however, is very fugitive and is apparently not produced by some aromatic thiolsulphinates.

We have found that addition of a solution of N-ethylmaleimide in *iso*propyl alcohol followed by potassium hydroxide in the same solvent to thiolsulphinates yields pink to red colours. Thiolsulphinates can therefore be distinguished from disulphides, which remain colourless. This is an extension of the colour test for thiols reported by Benesch and Benesch *et al.*[4]. The conditions for the test are essentially the same as described for thiols by these authors. The maleimide derivative and potassium hydroxide are used in dry *iso*propyl alcohol solutions, 0·05 *M* and 0·25 *M*, respectively, and the N-ethyl maleimide is used in a very large molar excess over the thiolsulphinate. The reaction may be applied on paper, in which case the paper containing a thiolsulphinate spot is first dipped in the N-ethyl maleimide solution, dried for 5–10 min., and is then dipped in the potassium hydroxide solution. A pink spot develops immediately where thiolsulphinate is present. The test may also be performed in solution in colorimeter tubes and absorbance or per cent transmittance measured.

For colorimetric analysis, 1 ml. of 0·05 *M* N-ethyl maleimide reagent is added to 0·5–5 μmoles of thiolsulphinate in 1 ml. of *iso*propyl alcohol and then 1 ml. of 0·25 *M* potassium hydroxide solution is immediately added. Intensity of colour is conveniently measured at 5150 A. (absorption maximum 5100 A.). The colour fades rapidly during the first 3 min. and then diminishes at a slower rate. Moderately reproducible results are obtained if the absorbance is measured at a specified time between 10 and 18 min. after the addition of alkali and compared with standard curves obtained from measurements of pure compounds. The intensity of colour varies somewhat with different thiolsulphinates. The reaction has been applied to several synthetic thiolsulphinates (RS(O)SR) where R is phenyl, *p*-tolyl, ethyl, *n*-propyl, and 2-propenyl. The purity of these compounds was established by infra-red analysis. An allicin isolate from garlic and a low-temperature vacuum distillate from a fresh onion concentrate (both of which were shown to be free of thiols) responded to the test. The reaction is sufficiently sensitive just to detect 5×10^{-2} μmole thiolsulphinate/ml. of solution.

In certain cases, the reaction will distinguish thiolsulphinates (RS(O)SR) not only from disulphides but also from thiolsulphonates (RSO$_2$SR). For example, *p*-tolyl *p*-toluene thiolsulphonate and phenyl benzene thiolsulphonate give no appreciable colour (indistinguishable from the background due to the reagents). Ethyl ethane thiolsulphonate produces a very faint colour, approximately 2 per cent of the intensity of the colour of the corresponding thiolsulphinate and *n*-propyl-*n*-propane thiolsulphonate gives a colour approximately 14 per cent as intense as that of the propyl thiolsulphinate. On the other hand, the colour produced by methyl methane thiolsulphonate is a third as strong as that of ethyl ethane thiolsulphinate (the corresponding methyl derivative was not available for comparison), although the compound was shown to be free of thiolsulphinate by infra-red analysis. The relatively high colour value given by the propyl thiolsulphonate compared with the corresponding ethyl compound may be a result of small amounts of thiolsulphinate as impurity in the former. Other classes of compounds responding to the test are thiols and thiolesters as shown by Benesch and Benesch *et al.*[4], and S-alkyl *iso*thiouronium bromides and aromatic sulphinic acids. In the spectrophotometric method for determining thiols[5] with N-ethylmaleimide in phosphate buffer, the thiol adds to the double bond of the maleimide as measured by the decrease in absorbance at 3000 A. In *iso*propyl alcohol, however, no addition is observed with aliphatic thiols or thiolsulphinates. Whether the addition of base produces a rapid addition to the double bond followed by other colour-forming reactions or whether the reactions follow a different course is not known.

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BIOCHEMISTRY

Direct Determination of Drug Concentrations in Biological Fluids by Polarography

It is an established fact that part of the initial testing of new drugs involves their estimation in biological fluids from animals or patients receiving the drug. This information is required to establish dosage regimes and to study rates and methods of elimination from the body. Hitherto, the use of physico-chemical techniques such as spectrophotometry, colorimetry and polarography has frequently required a separation of the drug from interfering materials present in the biological fluid. This communication suggests that, in suitable circumstances, the polarographic method can be successfully employed without any such separation.

During the clinical testing of the new anti-tubercular drug 2-ethyl-4-thiocarbamidopyridine (1314 *TH*) it became necessary to determine small concentrations (0·1–5·0 μgm./ml.) of the drug in serum and cerebrospinal fluid, and larger concentrations (>30 μgm./ml.) in urine from patients receiving the drug. Since 1314 *TH* gives a well-defined polarographic wave ($E_{1/2} = -0{\cdot}78$ volt vs. standard calomel electrode at *p*H 7·0) the utility of the polarographic method was investigated. (Full details of the electrochemistry of polarographic reduction of 1314 *TH* and of the methods described below for estimating its concentration in biological fluids will be the subject of further publications.)

It was found that, using a laboratory-constructed, pen-recording polarograph at its maximum sensitivity of 200 mm./μamp., de-gassed, normal sera of the rat, rabbit, horse and human were free from detectable polarographic waves over the potential range −0·1 to about −1·1 volts, using an internal silver wire anode. This potential range is conveniently referred to as the accessible region of potential. Normal rat and human urine were free from polarographic waves only over the region −0·2 to −0·7 volt. Moreover, the slope of the residual current and the oscillations due to growth and fall of drops were much greater than usual.

Accordingly, polarograms of known concentrations of 1314 *TH* in the above sera and cerebrospinal fluid were recorded and a well-defined wave of height proportional to concentration was obtained. There appeared to be no appreciable change of half-wave potential between serum solutions and aqueous solutions of the same *p*H. There was no need to add any extra supporting electrolyte or buffering agent beyond that already contained in the fluid. The limit of sensitivity with present equipment is ~0·2 μgm./ml. but it is hoped that this can be improved by suitable instrumentation. It was necessary to acidify the urine in order to bring the 1314 *TH* wave into the accessible region of potential, and addition of 5 per cent by volume of glacial acetic acid was a suitable procedure. The sensitivity was decreased to about 5 μgm./ml. because of the larger residual current. The polarographic method has been applied to serum, cerebrospinal fluid and urine from patients receiving 1314 *TH*.

Although most of the work on this problem has been devoted to the estimation of 1314 *TH* there seems no reason why any compound should not be estimated, the half-wave potential of which is in the accessible regions of potential and which does not interact significantly with serum proteins. Other compounds studied briefly in these laboratories to which the direct polarographic method can probably be applied are: pyrazine-2-carboxyamide, certain nitrofuran derivatives, *iso*nicotinylhydrazide and terramycin. The latter two drugs possess half-wave potentials close to the negative limit of accessible potential for serum, and some *p*H adjustment may be necessary to obtain highest sensitivities.

Since this preliminary communication was written, my attention has been directed to the work of Teisinger[1] on the direct polarographic determination of nitrobenzene in serum quoted by Brezina and Zuman[2]. It appears that the approach described above is not novel but has lain in abeyance for some twenty years.

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Effect of Azaserine (o-Diazoacetyl-L-serine) on the Pyridine Nucleotide Levels of Mouse Liver

The amount of diphosphopyridine nucleotide in mouse liver has been shown to increase upon the intraperitoneal injection of a number of pyridine derivatives[1,2]; nicotinamide is the most effective, raising the level 8–10 times. Although nicotinic acid is as effective as many of the analogues, it appears to inhibit the synthesis when administered with nicotinamide, as compared with nicotinamide alone. Inhibition of synthesis from nicotinamide injection has also been reported with the administration of the purine antagonists 6-mercaptopurine and thioguanine[1,3]. These inhibitions appear to affect only the level of diphosphopyridine nucleotide attained from nicotinamide injection and the rate of its subsequent return to the normal level, but do not appear to affect the basal level normally found in the liver.

Recently, the injection of azaserine has been shown to inhibit markedly diphosphopyridine nucleotide synthesis resulting from nicotinamide injection[4]. Unlike the effects of the other inhibitors of this system, the administration of azaserine alone causes a marked reduction in the normal levels of the pyridine nucleotide.

We have found that subcutaneous injection of azaserine at a dosage of 200 mgm./kgm. rapidly reduces the level of pyridine nucleotide in the liver. The diphosphopyridine nucleotide-levels in the livers of 2–3 months old (*BALB/cAN* × *DBA/2J*) F_1 hybrid mice were followed using the methylethyl ketone method previously described[1]. In Fig. 1, it can be seen that the pyridine nucleotide-level drops to a minimum value, which is about a nine-fold decrease from the level in control mice injected with 0·85 per cent saline, in 1–2 hr.

After reaching a minimum value, the pyridine nucleotide content was found to rise slowly over a 24-hr. period, approaching the level of the saline-injected, control animal. It appears that this dosage is somewhat toxic with approximately 25 per cent of the animals dying between 20 and 24 hr. after injection of azaserine.

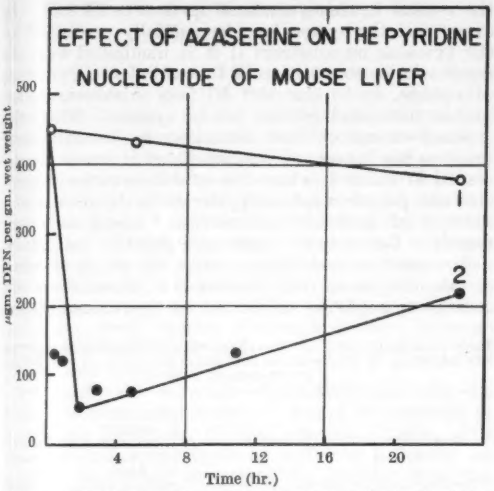

Fig. 1. Effect of azaserine on the pyridine nucleotide level of mouse liver. Determinations were made on a 5 per cent (w/w) trichloracetic acid extract of mouse liver. Each value represents the pooled samples of two mice. Curve 1, saline control; curve 2, azaserine-injected animals

Since the increase in diphosphopyridine nucleotide resulting from nicotinamide administration occurs mainly in the soluble fraction of the mouse liver, it was of interest to determine whether the azaserine reduction acts only on the soluble fraction. Accordingly, four animals were killed 1 hr. after azaserine injection, the livers homogenized in 0·25 *M* sucrose–0·05 *M* nicotinamide solution, pooled, and fractionated according to the method of Jacobson and Kaplan[5]. The results of this experiment, together with the values of the pyridine nucleotide content of livers of saline-injected animals treated similarly are compared in Table 1. Although the soluble fraction shows the greatest change in oxidized nucleotide content, the non-soluble fractions are lower, possibly indicating some equilibrium between the bound and soluble forms in the liver. There does not appear, however, to be an appreciable decrease in the reduced pyridine nucleotide.

Azaserine as well as 6-diazo-5-oxo-L-norleucine has been shown to inhibit synthesis of nucleotide precursors of diphosphopyridine nucleotide[6]. Accordingly, we have found that 6-diazo-5-oxo-L-norleucine behaved similarly to azaserine in lowering mouse liver diphosphopyridine nucleotide.

Inhibition of the precursors of pyridine nucleotide, either by the mechanism described above[6] or one similar to the system reported in red blood cells (also shown to be inhibited by azaserine[7]), would obviously result in a lowered diphosphopyridine nucleotide content of the liver. However, the rate of diphosphopyridine nucleotide destruction in mouse liver as indicated by radioactive experiments[4] cannot in itself account for the rapid decline of diphosphopyridine nucleotide that we have observed after azaserine administration. Furthermore, we have been unable to detect any destruction of diphosphopyridine nucleotide by azaserine under physiological conditions, nor have we observed increased activities of purified veal brain or *Neurospora crassa* diphosphopyridine nucleotidases when they were treated with azaserine. In fact the latter enzyme appears to be inhibited in the presence of high concentrations of azaserine.

Additional investigations are now being carried out in this laboratory in an effort to explain the above observations, and it is hoped that these studies will be of value in elucidating the pathway of diphosphopyridine nucleotide synthesis and its regulation in the intact animal.

Table 1. Distribution of Pyridine Nucleotides in Mouse Liver Homogenates

	μgm./gm. wet weight	
	Control	Azaserine injected
Oxidized		
Total homogenate	477	126
Nuclei	18·2	17
Mitochondria	23·7	12
Microsomes	14·8	8·2
Soluble	360	77·5
Reduced		
Total homogenate	260	198

Mouse livers were removed 1 hr. after azaserine injection. Controls were injected with saline. The values represent the pooled samples of four mice in each group.

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Reversal of Epinephrine Inhibition of Liver Tyrosine Oxidation

Thyroid hormone interacts with the rat liver tyrosine oxidase system *in vitro* and *in vivo*[1]. The site of the inhibition proved to be at the first enzymatic step of the system, namely, tyrosine-α-ketoglutarate transaminase[1,2]. Canellakis and Cohen[3] first demonstrated an inhibition of this enzyme by epinephrine, using the enzyme from dog liver. Since, under certain conditions, thyroxin and epinephrine are metabolically related[4], it seemed likely that a demonstration of a similar action of both these hormones on the same enzyme system might provide a basis for exposing a mechanism of hormone action. Some empirical effects of epinephrine upon blood amino-acid levels are known[5–7]; but virtually no attempts have been made to explain these results at the molecular level.

The effects of treating a soluble cytoplasmic tyrosine oxidase preparation from rat liver[8] with increasing

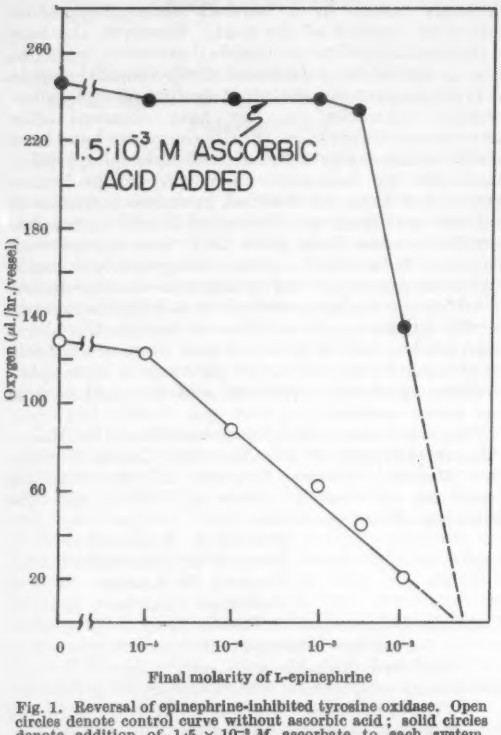

Fig. 1. Reversal of epinephrine-inhibited tyrosine oxidase. Open circles denote control curve without ascorbic acid; solid circles denote addition of $1{\cdot}5 \times 10^{-3}$ *M* ascorbate to each system. Epinephrine was incubated with the enzyme for 10 min. at 37° C. prior to addition of the substrate

concentrations of L-epinephrine showed that complete inhibition would occur at a level of 5×10^{-3} *M* epinephrine compared with a saturation-level of L-tyrosine co-substrate of about 2×10^{-3} *M*. Fractionation of the overall 5-step system into single, double, or triple enzyme sub-systems, using appropriate substrates[8], revealed that inhibition by epinephrine occurs only when tyrosine and α-ketoglutarate are metabolized compared with *p*-hydroxyphenylpyruvate or homogentisate oxidation-rates. This confirmed that the initial enzyme participates in the reaction[3]. Partially purified homogentisate oxidase[9], on the other hand, is usually stimulated by the addition of epinephrine, presumably due to a redox property of the hormone. Complete reversal of epinephrine inhibition of tyrosine oxidation is accomplished by $1{\cdot}5 \times 10^{-3}$ *M* ascorbate with as much as 5×10^{-3} *M* epinephrine added to the system (Fig. 1). The effectiveness of the vitamin decreases with higher concentrations of the hormone. It is unlikely that ascorbate functions by reducing the stability of epinephrine[10] to an appreciable extent in a system of this *p*H. It seems possible, however, that a chelate may be formed with an essential component and reversed by ascorbate although no evidence is available, as yet, on this point. Other redox agents which stimulate enzymes in the overall system, such as reduced glutathione[11] and dichlorophenolindophenol[12], fail to reverse the hormonal inhibition. Both norepinephrine and adrenochrome are inhibitors. Complete inhibition (extrapolation) occurs at 10^{-2} *M* L-norepinephrine. Only a slight reversal of norepinephrine inhibition is obtained by adding ascorbate to the system in concentrations up to 3×10^{-3} *M*. In every case the system can be partially protected by the tyrosine co-substrate if it is incubated with the enzyme prior to addition of the inhibitor. Pyridoxal phosphate, up to 5×10^{-3} *M*, fails to reverse epinephrine inhibition of the whole system. That the reversal of epinephrine inhibition by ascorbic acid involves the initial transaminase step is shown by the results in Table 1, where the oxidation-rates of tyrosine and *p*-hydroxyphenylpyruvate in the presence or absence of inhibitor and reversing agent are compared. Compounds such as ascorbyl palmitate, D-*iso*-ascorbate and ferrous ascorbate are as effective as ascorbic acid, but ferrous ion alone does not reverse epinephrine inhibition of the transaminase.

Table 1. Results of a Typical Manometric Experiment showing the Reversal of Epinephrine Inhibition to Occur at the Initial Transaminase Step

Substrate (6 μ*M*)	Addition	Corrected enzyme activity (μl. O_2/hr./flask)	Inhibition (per cent)
L-Tyrosine*		87	
,,	$1{\cdot}5 \times 10^{-3}$ *M* ascorbate (AA)	169†	
,,	1×10^{-3} *M* L-epinephrine (EP)	35	58
,,	AA + EP	162	
Keto-*p*-HPP		52	
,,	AA	123†	
,,	EP	50	0
,,	AA + EP	109	

* α-Ketoglutarate present; α-ketoglutarate not added when *p*-hydroxyphenylpyruvate (HPP) is substrate.

† The increase in activity due to addition of ascorbate results from the effect of ascorbate upon homogentisate oxidase (ref. 8).

Physiological significance of this interaction is suggested by *in vivo* experiments which show that intraperitoneal injections of L-epinephrine (1 mgm./kgm. body-weight for 1 hr.), compared with saline-injected rats, produce a 55 per cent inhibition of specific enzyme activity. Addition of $1{\cdot}5 \times 10^{-3}$ *M* ascorbate *in vitro* causes twice the stimulation of control specific activity in preparations derived from epinephrine-treated animals compared with saline-injected animals. Details and extension of this work will be presented elsewhere.

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Purification of Neuraminidase from *Vibrio cholerae*

Blix, Gottschalk, and Klenk[1] proposed the name 'neuraminidase' for the enzyme which splits the glycosidic linkage joining terminal (substituted or unsubstituted) neuraminic acid to residual oligo- or poly-saccharide. Kuhn and Brossmer[2] and recently Gottschalk and Graham[3] have isolated well-defined substrates by which the enzymatic action of neuraminidase could be studied. There exist many other substrates of high molecular weight and unknown structure; for example, mucoproteins from urine, saliva, serum, or stromata of erythrocytes. Neuraminidase is present in viruses of the myxo-group (influenza, fowl plague, Newcastle disease, mumps) and was found in filtrates of *Vibrio cholerae*[4] and *Clostridium welchii*[5].

To clarify the function of neuraminidase for the virus – host cell relationship it would be desirable to study the structure of the enzyme. Since virus material is not available in sufficient amounts an attempt was made to isolate the enzyme from the culture fluids of *Vibrio cholerae*.

In order to control the purification of the enzyme, a simple method for serial determinations was developed. The basis of this determination is the splitting of neuraminic acid (or its acyl derivative) from a standard substrate. Mucoprotein from human urine was used as standard, which was prepared by a modification of the method of Tamm and Horsfall[6]. Stromata of erythrocytes from sheep or calves were also found to be suitable.

The activity of neuraminidase was measured in the following way. 4 mgm. of urinary mucine containing 3·8–4·1 per cent neuraminic acid was dissolved in 1 ml. water. The enzyme solution (0·001–0·2 ml.) was added and the total volume made up to 2 ml. with 0·1 *M* acetate buffer at *p*H 5·5. This mixture was incubated for 15 min. at 37° C. The reaction was stopped by precipitation of the mucine with 2 ml. of a saturated solution of benzoic acid in chloroform (65 gm./100 ml.). After vigorous shaking, the solution was centrifuged at 3,500 r.p.m. for 4 min. The mucine forms a layer between the chloroform and the supernatant aqueous solution. From the clear supernatant solution 1 ml. was used for the determination of the liberated neuraminic acid by the orcin reaction according to Böhm, Dauber, and Baumeister[7]. A reference curve was obtained using crystalline methoxy-neuraminic acid, kindly supplied by Prof. E. Klenk. The quantity of the enzyme should be adapted in such a way that 8–20 μgm. of neuraminic acid are liberated. The unit *E* of enzyme activity was defined as that amount which liberates 1 μgm. of neuraminic acid in 15 min. at 37° C. under the conditions described. The purity of the enzyme is measured by the specific activity *E*/μgm. protein nitrogen. The nitrogen was determined by the Kjeldahl method if sufficient material was available; otherwise the preparation was hydrolysed by 6 *N* hydrochloric acid and the amino nitrogen was determined by ninhydrin by the method of Stein and Moore[8].

Filtrates of *Vibrio cholerae* cultures supplied by Behring-Werke, Marburg, were used as the starting material. The specific activity could be increased 50,000-fold by a relatively simple procedure. At the beginning of this work, enzyme instability caused certain difficulties. The purified enzyme sometimes lost its activity within a few hours. Sufficient stabilization could be achieved, however, by employing chelating agents. Addition of cyanide was particularly effective during purification, but the enzyme can also be stabilized by serum albumin and other proteins.

Table 1. PURIFICATION OF NEURAMINIDASE

Fraction	Activity (*E*/ml.)	Specific activity (*E*./μgm. nitrogen)	Purification factor
1. *Vibrio cholerae* filtrate	30–60	0·01–0·03	1
2. Concentrate after elution from erythrocytes	200–300	1–2	100
3. Eluate from column chromatography	300–500	120–130	6,000
4. After precipitation with ammonium sulphate	20,000–100,000	1,000	50,000

The first step in purification was the concentration of neuraminidase by adsorption to sheep erythrocytes at *p*H 8–9, followed by an elution at *p*H 5·5, in a way similar to that described by Burnet[4]. Since hæmolysis cannot be avoided, the preparation is heavily contaminated with hæmoglobin.

The following step is the most significant. It may also be useful for the purification of other enzymes which have a high affinity for their specific substrate. This step combines the specific binding of the enzyme to its substrate with column chromatography. Stromata of erythrocytes from sheep or calf, prepared by two-fold hæmolysis, were used as substrate. They were mixed with kieselgur (Merck) to form a chromatographic column. The enzyme was adsorbed at 0° C., *p*H 9 in a buffer containing 0·01 *M* borate, 0·9 per cent sodium chloride and 0·1 per cent calcium chloride. The contaminating hæmoglobin and other proteins were washed out with the same buffer, and the enzyme was eluted at *p*H 5·5 at 0° C. In the adsorption buffer, the enzyme is firmly bound to the substrate, whereas in the acidic medium the substrate is split and the enzyme released.

The eluate from the column was concentrated over phosphorus pentoxide after addition of potassium cyanide. For further purification, the enzyme can be precipitated from the concentrate by half saturation with ammonium sulphate. On the other hand, if a solution containing a lower concentration of ammonium sulphate is slowly evaporated, a protein containing the total enzyme activity is crystallized out in needles.

The concentration of the enzyme and the increase in specific activity during the purification process is shown in Table 1. The purification was repeated several times. In each step of the procedure, 70–80 per cent of the activity was recovered. From the values in Table 1, it may be calculated that the starting material contains 50 μgm. enzyme nitrogen or 300 μgm. enzyme protein per litre. 12 l. of *Vibrio cholerae* filtrate were used in order to obtain sufficient material for ultra-centrifuge studies.

The purified protein is sedimented down with a fairly homogeneous boundary. A sedimentation constant of about $s_{20} = 1·3$ S. was measured at *p*H 8·5 in a solution containing 0·02 *M* potassium cyanide. From this value a molecular weight of 10–20,000 is estimated, assuming a spherical shape. Boundaries with higher sedimentation constants were observed under other conditions, suggesting that the protein has a tendency to aggregate. Evidence for the identity of the isolated protein with the enzyme is based on the high specific activity of the

protein and the specificity of the purification procedure. Furthermore, it was found that the sedimentation of enzyme activity, measured by the decrease in enzyme activity in the supernatant, was of the same order as the sedimentation constant of the boundary in the ultracentrifuge. Finally, in the purified preparations, proteinases were no longer detectable.

The reaction kinetics of the enzyme was studied in some detail. A Michaelis constant of about $1–2 \times 10^{-4}$ mole neuraminic acid/l. was determined using sheep stromata as substrate. This confirms the high affinity of the neuraminidase for the specific substrate, which made possible the specific purification procedure described here.

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Asymmetric Hydrolysis of Diethyl Acetamidomalonate by Chymotrypsin

Continuing our study[1] of asymmetry in reactions of non-asymmetric molecules of the type *Ca,b,d,d*, we are examining the hydrolysis of diethyl acetamidomalonate $CH_3CONHCH(COOC_2H_5)_2$ by chymotrypsin. The ester (Matheson) was crystallized from benzene–petroleum ether, m.p. 96·5–98° C. Chymotrypsin was from Worthington, salt free, lot *CD* 576–81. The kinetics of hydrolysis were studied in water at 25°, with magnetic stirring, in the *p*H range 7·0–7·95 with *tris*hydroxymethylaminomethane ($3·3 \times 10^{-3}$ *M*) or phosphate ($8·3 \times 10^{-3}$ and $1·6 \times 10^{-2}$ *M*) buffers. Runs were carried out with a Beckman model *G* *p*H meter, 0·1 *N* sodium hydroxide being added from a Gilmont micro pipette-burette to maintain the *p*H, or, at high enzyme concentrations, with a *p*H-stat, radiometer titrator and automatic burette delivering 0·4 *N* sodium hydroxide.

The hydrolysis was first order in the ester and the rate 'constants' k_1 were proportional to enzyme concentration (Table 1). The reaction stopped after liberation of one equivalent of acid. The ester was quite stable in the absence of enzyme, its hydrolysis in $3·3 \times 10^{-3}$ *M* buffer at *p*H 7·8 showing an apparent *k*, of 0·0004 min.$^{-1}$. The hydrolysis is first order in both substrate and enzyme under these conditions.

The product of one run, 20 ml. initially 0·02 *M* in the ester was dialysed against 0·001 *N* hydrochloric acid and this was extracted with ether

Table 1. Hydrolysis of Diethyl Acetamidomalonate by Chymotrypsin at *p*H 7·8 ± 0·15

Chymotrypsin (mgm./ml.)	Ester (*M*)	k_1 (min.$^{-1}$)
0·39	0·0046	0·016 ± 0·001
0·39	0·0092	0·016 ± 0·001
0·39	0·0200	0·016 ± 0·001
0·50	0·0200	0·020
0·10	0·0200	0·042
1·69	0·0637	0·067

leading to 2-acetamido-2-carbethoxyacetic acid $CH_3CONH—C(COOH)(H)—COOC_2H_5$, 0·053 gm., 70 per cent yield; m.p. from benzene–ethanol 138–139° dec.; mixed m.p. 139–141°, synthetic sample 140–141°, reported[2] 140–141·5°.

The product of a run, carried out in the *p*H-stat at 7·8, with 0·05 *M* ester, an enzyme concentration of 0·91 mgm./ml., and 0·0069 *M* phosphate, was examined in a Zeiss–Winkel polarimeter after 3 hr. reaction, one equivalent of acid having been generated, and was found to be optically active, $\alpha_{obs.} + 1·77° \pm 0·03$ (after minor correction for chymotrypsin), $[\alpha]_D^{22} + 63° \pm 2$ for the half-ester, based on its presence in quantitative yield as one enantiomorph (the value is not inconsistent with those reported for some derivatives of monosubstituted malonic acids[3]). The rotation fell with time reaching half its original value in about 44 hr. The product of another run carried out with 0·016 *M* phosphate and not examined as quickly led to $[\alpha]_D^{22}$, + 59°. When the hydrolysis was followed in the polarimeter, necessarily in more concentrated (1 *M*) buffer, the rotation rose initially ($\alpha_{obs.}$, + 1·55°) and then fell in 4 hr. to the reading of the blank.

The ester, unlike the related diethyl benzylmalonate, which is rather inert[4] to chymotrypsin under comparable conditions, is hydrolysed by this enzyme to the half-ester. The observed optical activity indicated that this enzyme distinguishes between the two similar ester groups in diethyl acetamidomalonate and that the half-ester is formed optically active and racemized during isolation, presumably by enolization (this kind of steric selectivity has been demonstrated by use of tissue homogenates for citric acid[5] and for glycerol[6]). Isolation of the optically active half-ester is now being attempted. These experiments indicate that the related aminomalonic acid, which is decarboxylated enzymatically[7], may be found to do so asymmetrically, as has been suggested[8], and as would be required by the results of conversion of serine, labelled isotopically in nitrogen and carboxyl carbon, to glycine[9], if aminomalonic acid is to be an intermediate in this process. This is indicated also by reactions of methylmalonic acid[10].

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Reduced Diphosphopyridine Nucleotide—Neotetrazolium Reductase

MARTIUS[1] has reported the presence in animal tissues of a quinone reductase (phylloquinone reductase) similar in properties to menadione reductase described by Wosilait and Nason[2]. Phylloquinone reductase couples the oxidation of reduced diphosphopyridine nucleotide (DPNH) with the reduction of vitamin K_3. Since the reduced form of vitamin K_3 reduces neotetrazolium chloride (NT) directly to the coloured insoluble formazan[3], it is of interest to investigate the production of formazan by the enzymic oxidation of reduced diphosphopyridine nucleotide in order to ascertain if this enzymic sequence involves a quinone reductase. In this communication the enzymic process linking the oxidation of reduced diphosphopyridine nucleotide with reduction of neotetrazolium chloride is termed DPNH–NT reductase.

Liver homogenates were prepared either in water or in 0·25 *M* sucrose at 0° C., using a conventional Potter–Elvehjem type homogenizer. The animals used were adult female rats of the Medical Research Council strain, body-weight 180–200 gm.

DPNH–NT reductase was assayed by incubating aerobically 0–20 mgm. wet weight tissue with 1 ml. phosphate buffer (*p*H 7·4, 0·1 *M*), substrate (reduced diphosphopyridine nucleotide, or diphosphopyridine nucleotide plus β-hydroxybutyrate) and 0·15 ml. of 1 per cent neotetrazolium chloride. Buffer, tissue and substrate were pre-incubated for 2 min. at 37° C. aerobically before the addition of neotetrazolium chloride. The reaction was stopped by the addition of 1 ml. of 10 per cent trichloracetic acid; the formazan was extracted with 4 ml. of ethyl acetate. The extinction at 510 mμ was obtained using a Hilger 'Uvispek' spectrophotometer. When other additions were made to the reaction mixture (see Table 1), the reagent added was usually incubated with buffer plus tissue for 15–30 min. at 0° C. Except in those experiments involving differential centrifuging all tissue suspensions were prepared in water to reduce the possibility of permeability phenomena arising with mitochondrial enzymes.

The addition of reduced diphosphopyridine nucleotide (0–1·0 μmole) or of diphosphopyridine nucleotide (0–1·0 μmole) plus β-hydroxybutyrate (final concentration 0·01 *M*) to the incubation mixture led to rapid production of formazan with liver suspensions (Fig. 1). The effects of adding vitamins K_1 and K_3 are also shown in Fig. 1; both vitamins were added in suspension as described elsewhere[4].

Table 1. ACTION OF VARIOUS INHIBITORS, UNCOUPLING AGENTS, QUINONES AND VITAMINS ON DPNH–NT REDUCTASE

Substrate	Addition (in 0·1 ml.)		Formazan production as percentage of control value
	Substance	Amount (μmoles)	
DPN+BHB*	amytal	2·0	102
DPNH	amytal	12·8	94
DPNH	QMS	5·0	97
DPNH	QMS	10·0	78
DPNH	QMS (anaerobic)	10·0	84
DPNH	quinine sulphate	1·0	100
DPNH	azide	20·0	97
DPNH	versene	20·0	93
DPNH	α,α′-dipyridyl	2·0	92
DPNH	α,α′-dipyridyl	25·0	98
DPN+BHB	manganous chloride	0·4	60
DPNH	manganous chloride	0·4	65
DPN+BHB	2.4-dinitrophenol	0·2	75
DPNH	2.4-dinitrophenol	0·1	95
DPN+BHB	dicoumerol	1·2	43
DPNH	dicoumerol	1·2	20
DPNH	1.4-naphthaquinone	2·0	7
DPN+BHB	vitamin K_3	1·0	21
DPNH	vitamin K_3	1·0	45
DPN+BHB	vitamin K_1	0·3	182
DPNH	vitamin K_1	0·6	111
DPNH	PCMB	1·0	5

* BHB, β-hydroxybutyrate.

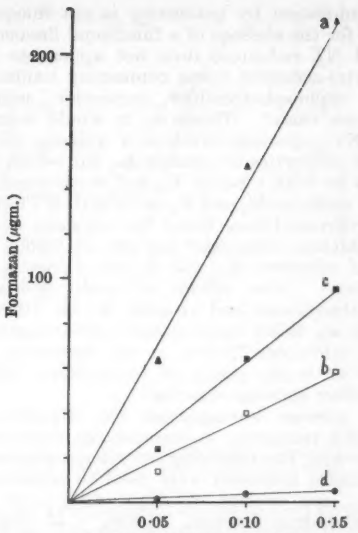

Fig. 1. Formazan production by rat liver tissue suspensions; diphosphopyridine nucleotide + β-hydroxybutyrate as substrate. Curve (*a*), 100 mgm. wet weight tissue/ml.; 1 μmole diphosphopyridine nucleotide; incubation, 10 min. Curves (*b*), (*c*) and (*d*), mitochondrial suspension (1 : 5) frozen and thawed three times; incubation, 15 min.; diphosphopyridine nucleotide, 0·5 μmole; further additions: curve (*b*), 0·1 ml. phosphate/ethanol/plasma albumin suspension; curve (*c*), 0·1 ml. vitamin K_1 (0·3 μmole added) in phosphate/ethanol/plasma albumin; curve (*d*), 0·1 ml. vitamin K_3 (0·8 μmole added) in phosphate/ethanol/plasma albumin

Previous reports have indicated that enzymic reduction of tetrazolium salts occurs via a flavoprotein[5]; in an attempt to investigate this possibility the effects of several types of inhibitor were studied. The results are shown in Table 1. It can be seen that sodium amytal, quinine sulphate, α-α′-dipyridyl, sodium azide and sodium versenate had no appreciable effect on DPNH–NT reductase. Quinacrine methane sulphonate (QMS) was inhibitory only in high final concentration both under aerobic and anaerobic conditions. The sulphydryl-reagent *p*-chloromercuribenzoate (PCMB) was strongly inhibitory, as were quinones like 1.4-naphthaquinone, vitamin K_3, and the natural quinone antagonist dicoumerol. Vitamin K_1 was, however, stimulatory in low final concentration. DPNH–NT reductase was inhibited in a variable manner by manganous chloride and by 2.4-dinitrophenol, both of which are known to uncouple oxidative phosphorylation. 2.4-dinitrophenol has also been reported to inhibit quinone reductase and menadione reductase[2].

The results suggest that DPNH–NT reductase, which is an extremely active enzyme in rat liver, does not contain an easily dissociable flavoenzyme complex which would presumably be strongly inhibited by quinine sulphate or quinacrine methane sulphonate. Wosilait and Nason[2] reported that quinacrine produced no inhibition on menadione reductase. It would appear then that if DPNH–NT reductase contains a functional flavoenzyme the flavine group is strongly bound to the protein. A

lack of inhibition by quinacrine is not unequivocal evidence for the absence of a functional flavoenzyme.

DPNH–NT reductase does not appear to utilize the amytal-sensitive route connecting oxidation of reduced diphosphopyridine nucleotide with the cytochrome chain[6]. However, it would seem that DPNH–NT reductase involves a quinone structure similar in properties to vitamin K_1 and which can be inhibited by both vitamin K_3 and dicoumerol. The effects of vitamins K_1 and K_3 on DPNH–NT reductase are the reverse of those found for succinate–neotetrazolium chloride reductase[4] but are analogous to the effects of vitamins K_1 and K_3 on oxidative phosphorylation[7]. The effects of such quinones as 1.4-naphthaquinone and vitamin K_3 on DPNH–NT reductase are being investigated further to ascertain if their inhibitory power is an unspecific redox property or is the result of competitive inhibition with another quinone structure.

Using sucrose homogenates the distribution of DPNH–NT reductase in intracellular fractions was investigated. The following centrifugal accelerations for the times indicated were used to separate each fraction: (*a*) nuclear fraction, $600g \times 10$ min.; (*b*) mitochondrial fraction, $10{,}000g \times 15$ min.; (*c*) microsomal fraction, $105{,}000g \times 30$ min. The reductase appears to be largely associated with the mitochondrial and microsomal fractions. In a typical experiment the percentage of the total activity associated with each fraction was: nuclear, 8; mitochondrial, 60; microsomal, 28; and soluble fraction, 3.

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Carotene Content of *Ulex europaeus* (Common Furze)

A general investigation has been in progress for some time in this Department into certain aspects of the chemistry of *Ulex europaeus*. This common furze has a local reputation as a supplementary animal foodstuff, while it has also been reported from France as a suitable fodder for sheep[1].

The analytical results (mean percentages) from air-dried samples of furze growing on waste land in County Cork during the past year are as follows: moisture, 18·4; crude protein, 10·2; fibre, 26·6; fat, 1·2; nitrogen-free extract, 41; inorganic matter, 2·6.

No previous results are available on the carotene content of the green matter (spines) of furze and its seasonal variation, which are now reported. The following procedure has been followed in making the determinations on fresh plant material.

The furze bushes were collected in the mornings; the green, prickly spines were taken for analysis and the main woody portion of the plant discarded. The spines were chopped as soon as possible after picking. Representative samples were blanched for 1 min. in boiling water[2] and stored in a frozen condition when analysis was not immediately undertaken. Losses of carotene of up to 15 per cent were recorded when unblanched samples were analysed after 1 hr. storage. The procedure also facilitated extraction of the pigments.

The furze samples were treated with a 3 : 2 mixture of acetone–petroleum ether (boiling point, 40–60° C.) for 2 min. in a high-speed 'blendor' fitted with a small baffle-plate[3]. Calcium carbonate, just sufficient to neutralize plant acids, was added[4]. The extract was filtered, the entire process repeated twice more and acetone washed from the bulked solvent solution with water. Three processings were found to be sufficient as analysis of residues yielded no pigments.

The petroleum ether solutions were chromatographed on 10 cm. × 1·5 cm. columns of either, (1) a mixture of equal parts of magnesium oxide ('Sea Sorb 43' Fischer) and 'Hyflo-Super Cel' Johns-Manville[5], or (ii) alumina[6]. In both cases the columns were topped by a 2-cm. layer of anhydrous sodium sulphate. Carotene was eluted with 4 per cent ether in petroleum ether. Elution could be followed visually and its completion readily gauged. The eluate was made up to volume and its absorption measured on a Beckman spectrophotometer (model *D.U.*). Control analyses showed quantitative recovery of carotene added to furze samples.

The variation in carotene content of furze over a year's survey is shown in Table 1.

Table 1

Month	Carotene (mgm./kgm. dry weight)	Month	Carotene (mgm./kgm. dry weight)
January	124	July	253
February	125	August	230
March	134	September	138
April	159	October	147
May	171	November	120
June	235	December	101

Separation of the carotene mixture showed that β-carotene was the main constituent, up to 95 per cent of the total.

From October to February or March—when furze is fed to stock—the average carotene content is 126 mgm./kgm., indicating that it is a very good, and freely available, source of carotene. The relatively high values during the summer months point to the possibility of using dried furze meal as a supplementary source of carotene in animal feeding.

The problem of retention of carotene in dried furze is being investigated concurrently with that of the amino-acids in the furze protein, and a detailed report will be published later.

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PHYSIOLOGY

Elongation of Rat-Tail Tendon on Hydration

THE extent of hydration in connective tissues is of considerable physiological importance. Water retained by mucopolysaccharides and collagen is thought to have a pronounced effect upon the mechanical functioning of the tissue[1]. The rate and extent of swelling of collagen[2] were studied in order to measure the hydration of collagen as well as to ascertain how this might be related to the degree of cross-linking of the protein. The objective of the work discussed here was to analyse the rate and extent of elongation of a dry tendon when it was immersed in solutions.

Tendons were removed from rat tails, after stripping away the skin, by the usual method of blunt dissection with a pair of pliers. They were washed three times in 50 ml. of 0·1 *M* sodium chloride solution then washed continuously with distilled water for 30 min. Within half an hour of exposure to a temperature of 22–25° C., the tendons dehydrated to a constant ratio (W) of weight/length. A single tendon was inserted in a 10-ml. syringe cylinder through the bore of the glass tip; one end was immobilized by forcing a three-way adapter over the tip while the other end, 10 cm. distant, was attached to a mechanical lever system by a small 'Alligator' clip. A 70-cm. lever arm having a mechanical ratio of 20 was used to record the elongation on an electrically driven kymograph. The load on the tendon was 20 gm. throughout the entire process.

Upon the addition of water the length increased from an initial 'dry' value (L_0) to a steady-state value (L_0+L_∞) in about 1 min. At this point the water was removed and air was sucked through the cylinder. The tendon was allowed to dry for 20 min. at a constant temperature while it shortened to (L_0) with a change in length (L_∞) of 2–4 mm. A base line was then drawn on the kymograph, the elongation on hydration was restarted by adding water, and the increase in length recorded continuously until the steady-state length (L_0+L_∞) was reached. The process of elongation on hydration and contraction on dehydration could be reproduced at least 10 times without any apparent alteration in the response of the tendon.

The length – time curve for a typical hydration–elongation is shown in Fig. 1. The first derivative ($\Delta L/\Delta t$) is plotted on the same graph to show the relationship between a point of inflexion in the displacement curve and what might be a discontinuity in the velocity curve. When the first derivative was plotted against the quantity ($L_\infty - L$) a curve resulted similar to that obtained when it was plotted against t. When the first derivative was plotted against the quantity ($L_\infty - L)t$ a linear relationship was found:

$$dL/dt = K(L_\infty - L)t \qquad (1)$$

Here, dL/dt is the rate of change of the length of tendon during the hydration, K is a specific rate constant for the process, L_∞ is the maximum change in length attained, and L is the change in length at any particular time, t. Rearrangement of the terms in equation 1, followed by integration between the limits from zero to L in the time-interval from zero to t, gives equation 2:

$$\log (L_\infty - L) = \log (L_\infty) - Kt^2/(2 \times 2{\cdot}303) \qquad (2)$$

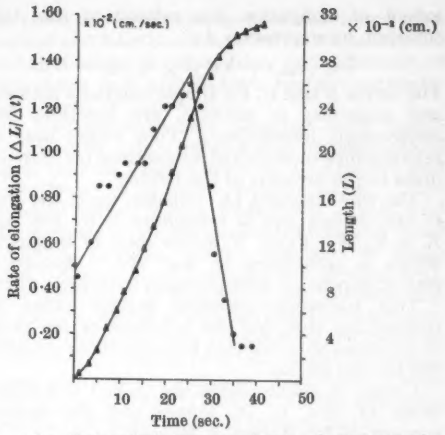

Fig. 1. The displacement (curve) and rate of displacement (straight lines) of a tendon during the hydration-elongation process. Water temperature is 23° C. and load is 20 gm.

Fig. 2 shows a family of curves plotted according to equation 2. The elongation on hydration of the same tendon was performed in water/methanol solutions where the composition was changed from 0 to 80 per cent methanol in steps of 10 per cent. The equation was obeyed satisfactorily for the elongation on hydration induced by the addition of water. However, mixed solvents produced an initial non-linear decrease in the term $\log (L_\infty - L)$ which after a short interval became linear with t^2. The extent of the deviation was minimal in water/methanol solutions but increased for other mixed solvents in the order methanol < ethanol < *iso*-propanol and propanol. The rate constant (K) was evaluated from the linear portion of the curve of the results with water/methanol shown in Fig. 2. Equation 3 shows a relation which was found to exist between K and the dielectric constant (D) of water/methanol solutions:

$$\log K = \log K_0 + B(D - 1)/(2D + 1) \qquad (3)$$

At concentrations of methanol greater than 40 per cent there was a positive deviation of the results indicative of specific solvent effects. It was found also that, over the region 0–80 per cent methanol, the

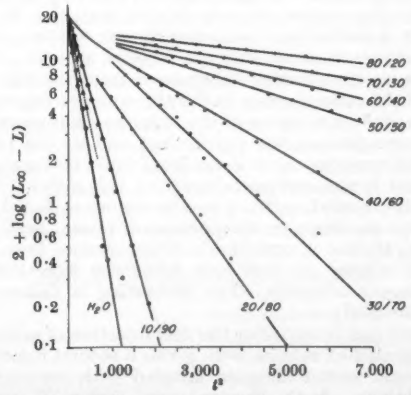

Fig. 2. The hydration-elongation of a tendon by water/methanol solutions ranging from 0 to 80 per cent methanol. The dotted line is a re-run of the water control after an 80 per cent methanol run. The initial non-linearity is shown as a curved line in an exaggerated form for the 40/60 composition

extent of elongation was related to the dielectric constant by expression 4:

$$\log (L_\infty) = \log (L_\infty)_{D=\infty} - C/D \qquad (4)$$

The terms B and C, for simple reactions between ions and molecules in solution, are functions of their electrostatic interaction[2]. They might bear similar relationships to electrical interactions between charged units in the proteins of the tendon.

The rate constant (K) depends upon the diameter of the tendon and is related to it by the equation $K = K_0/W$. Since W is the ratio of weight/length, which is equivalent to $4\pi r^2 \times L \times$ density$/L$, the rate of elongation depends upon force/area, or tension.

This mechanico-chemical system offers a new method for studying the influence of the properties of solutions on a reaction between these solutions and the rat-tail tendon.

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Drug-induced Immunological Tolerance

Previous work in this laboratory demonstrated that administration of a purine analogue, 6-mercaptopurine, suppressed the antibody response to a soluble antigen (human serum albumin) in rabbits[1]. It was shown that the antimetabolite could block completely the primary immune response to the purified protein antigen; its effect on the secondary response, however, was minimal[2]. It is the purpose of this communication to describe the apparent induction of immunological tolerance in adult rabbits by the use of 6-mercaptopurine.

Adult New Zealand rabbits were given one intravenous injection of human serum albumin labelled with iodine-131; the disappearance of the labelled antigen from the plasma was determined by the method of Talmage *et al.*[3]. In the experimental group a daily intramuscular injection of 6-mercaptopurine (6 mgm./kgm.) was given beginning with the day of antigen injection and continued for two weeks. Control animals received only the labelled antigen. The human serum albumin disappeared from the circulation of the control group in the three phases described previously[2]; the onset of the immune disappearance phase occurred on the eighth day, and by the tenth day radioactivity (that is, antigen) could no longer be detected in the serums taken from this group. The animals treated with 6-mercaptopurine failed to develop an immune disappearance phase, and the antigen showed a logarithmic disappearance from the serum, similar to that seen after the injection of homologous albumin, thus indicating a failure to form antibody.

About one month after the first injection of antigen, both groups of rabbits were given a second injection of human serum albumin labelled with iodine-131. In addition, both groups were given 20 mgm. unlabelled bovine γ-globulin intravenously. No further 6-mercaptopurine was given. The control group developed a typical secondary response to human serum albumin (Table 1) and precipitating antibodies to human serum albumin and bovine γ-globulin appeared in their serums. The drug-treated group again showed a normal or logarithmic disappearance of labelled human serum albumin without any evidence of antibody formation to this antigen. Precipitating antibodies to bovine γ-globulin, however, were present in their serums.

Table 1. Antibody Levels in Drug-induced Immunological Tolerance (μgm. N/ml. Serum)

		Day of experiment		
		14	48	80
Controls	Anti-human serum albumin	62	256	354
	Anti-bovine γ-globulin	—	107	288
Tolerant	Anti-human serum albumin	0	0	0
	Anti-bovine γ-globulin	—	94	242

About one month following the second injection of antigens, both groups were given a third injection of labelled human serum albumin together with a second injection of unlabelled bovine γ-globulin. Again, no 6-mercaptopurine was given. Both groups developed typical secondary responses to bovine γ-globulin. The human serum albumin was rapidly removed from the circulation of the control group, indicating the presence of humoral antibody to human serum albumin at the time of the third injection of that antigen. The group treated with the drug, however, again showed no evidence of anti-human serum albumin formation, as determined by the logarithmic disappearance of the labelled serum from the circulation, and by negative precipitin tests.

The term 'actively acquired tolerance', first used by Billingham, Brent and Medawar[4], was defined as "an induced, specific, central failure of immunological response brought about by an exposure of animals to antigenic stimuli *before maturation of the faculty of immunological response*" (italics ours). Tolerance of this type to homografted tissues and organs as well as to simpler antigens has been induced in laboratory animals by many workers[5]. In all such experiments, this has been carried out in the newly born or immature animal. The mechanism of this phenomenon is obscure; but the results of the present study indicate that the fœtal state is not essential for its induction. As a working hypothesis, we propose that the metabolic activity provoked in antibody-forming cells by antigenic substances, in addition to providing

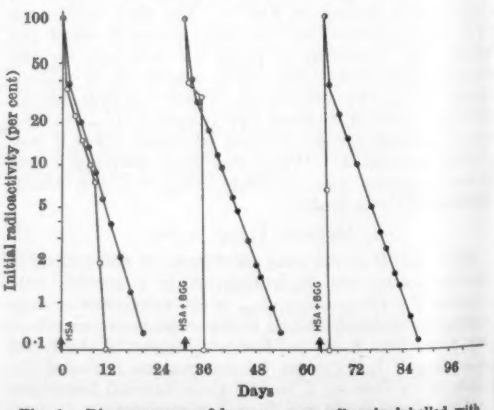

Fig. 1. Disappearance of human serum albumin labelled with iodine-131 in control and tolerant rabbits. ○, Controls; ●, animals treated with 6-mercaptopurine

the mechanisms needed for the production of antibody globulin, sets up an 'information storing device'. It is evident that this double metabolic response can be blocked by an antimetabolite. In the case of the fœtus or the newly born, the enzymes participating in the immune response may be under-developed. In the drug-treated animals it is apparent that although antibody production in general is not blocked, a gross dysfunction of the information-storing device has occurred, since there is no longer a 'recognition' of the previously administered heterologous antigen as a foreign material. Antigen is now metabolized along presumably normal pathways, similar to those in which autologous substances are catabolized. It is possible that the cells concerned in these pathways, like those which participate in the immune response, may also store information, perhaps in the form of a nucleic acid code, so that on re-exposure to antigen or to autologous material, immunologically tolerant animals again disposed of them along the previously utilized routes. These metabolic avenues of autologous or normal degradation, in contrast to those of the immune-response, appear to be resistant to 6-mercaptopurine and may be fully developed in immature animals. In any event, these experiments indicate that the term 'acquired immunological tolerance' previously used only for the response of the immature animal needs to be broadened to include drug-induced tolerance.

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Anticoagulant Action of Fibrin Surfaces on Mammalian Blood

MANY foreign surfaces have been studied in contact with blood in efforts to find out whether they might act as physical anticoagulant agents. Recently, a blood-born surface made of fibrin has been introduced in our laboratory in a number of hæmorheological studies, based on a concept of one of us (A. L. C.) which proposes that an endo-endothelial fibrin film is being formed and maintained throughout life in all blood vessels[1]. These studies demonstrated that the apparent viscosity and wall adherence are markedly decreased in blood in contact with fibrin surface when compared with different foreign surfaces, suggesting that, physiologically, the endo-endothelial fibrin film may constitute a major factor in aiding circulation, for example, by increasing the velocity of flow of blood[1,2]. We therefore undertook a comparative study of the coagulation of undiluted native blood in contact with fibrin and foreign surfaces and an account of the unexpected results is presented.

Special precautions were taken to avoid any admixture of tissue juice when securing the blood sample, particularly since it has been shown by Lalich and Copley[4] and later confirmed[5,6] that the admixture of minute amounts of tissue juice, by its content of thromboplastin and geloplastin[7], hastens markedly the coagulation of native blood.

Fibrin surfaces have been obtained from native blood, recalcified citrate plasma and preparations of fibrinogen (bovine plasma fraction I, Armour) and thrombin (Maw) as so-called 'fraction fibrin'. The procedures of coating 'Pyrex' glass tubes, 11 mm. × 50 mm., with different foreign surfaces, fibrin and other blood protein surfaces will be described in detail elsewhere. Native blood was properly secured from the antecubital vein of healthy human subjects, the jugular vein of cows and pigs, and the rabbit's heart. Needles and glassware were all siliconed for handling native blood or plasma until it was placed for testing into tubes with various surfaces. Experiments were run at 21° C. and also at 1° C. and 37° C., the formation of a complete gel being taken as the end-point.

In all tests, native plasma fibrin surface from blood of humans, cows, pigs and rabbits, showed always the longest and nearly identical coagulation times when compared with the silicones (*MS* 200/1000 and *MS* 1107), 'Perspex', paraffin wax, 'Lusteroid' and glass. Our siliconed tubes gave similar blood coagulation times of about half an hour, as originally described at 20° C. by Jaques *et al.*[6] for dogs, rats and men. In some cases, native plasma contacting native plasma fibrin was not coagulated after one week in the refrigerator at 1° C. On fibrin surfaces, native plasma gave much longer coagulation times than native blood. At 1° C. a hypercoagulable rabbit blood had plasma coagulation times of 45 min. on glass, 75 min. on silicones and 110 min. on human and cow native fibrin.

Observations were made on adherence and retraction of blood coagula in contact with human, cow, pig, and rabbit plasma fibrin surfaces, compared with silicone and glass surfaces. Silicone surfaces showed most adherence and least retraction, while native fibrin surfaces exhibited least adherence and most retraction. These findings may be compared with coagulation thrombi, in dogs[8], demonstrating that a coagulum which forms within a non-infected artery or vein is synergetic and non-adherent to the vessel wall and therefore of potential danger for the genesis of thromboembolism.

Table 1 shows a comparison of human and rabbit blood coagulation times from the same blood withdrawal at 21° C. on surfaces of native rabbit plasma fibrin, bovine fraction fibrin, and coatings of rabbit serum and bovine fibrinogen. This experiment proves that, although films of fibrinogen and serum proteins have an anticoagulant action, hitherto unknown, fibrin surface appears to act as a specific physical anticoagulant. In the course of preliminary studies, fraction fibrin-coated glass tubes were subjected to a temperature of 100° C. several times for 2 min. and thereafter 1 ml. of blood was put into them, with the

Table 1. THE COAGULATION OF HUMAN AND RABBIT BLOOD IN CONTACT WITH FIBRIN AND OTHER SURFACES

	Blood coagulation times at 21° C.	
	Rabbit (XVI) (min.)	Human (D. S.) (min.)
Native rabbit plasma fibrin	38	36
Bovine fraction fibrin	60	31
Rabbit serum	28	24
Bovine fibrinogen	28	24
Glass ('Pyrex')	10	13

result that the blood failed to coagulate at all. Transfer of blood or plasma from such tubes into glass tubes did not alter this condition; however, the addition of thrombin always resulted in coagulation. This experiment was repeated a number of times and forms the subject of another publication.

The unexpected findings with surfaces fibrinized at room temperature have a direct bearing on many fundamental problems in blood coagulation research, since initiation of coagulation has thus far been studied only on surfaces foreign to blood, whereby glass surface particularly was found to activate certain plasma coagulation factors[9,10]. New questions are being posed about the initiation of intravascular coagulation where the surface contacting the circulating blood may well be the endo-endothelial fibrin film.

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Submicroscopic Organization of Post-synaptic Membranes

Because of their importance in the transmission of impulses, the postsynaptic membranes have been repeatedly investigated by histochemical and electron-microscopical methods. Very little is known, however, about the internal organization of these structures. It was felt that polarization microscopy might give useful information in this respect.

An investigation was carried out on the thoraco-humeral muscle of the frog. According to Couteaux[1], the postsynaptic membranes of this muscle consist of a pallisade-like arrangement of minute lamellæ (3–5μ in length) situated transversely to the nerve end-branchings. It was found that these lamellæ exhibited no birefringence either in fresh frozen sections or in fixed material. On micro-dissecting living muscle-fibres in frog's Ringer solution, a slight birefringence was detected in the synaptic areas, which, however, disappeared in a few seconds after the cover-glass had been placed upon the preparation. This observation led to the assumption that the submicroscopical organization of these structures is very labile—a fact which may be a pre-condition of the synaptic function.

Fig. 1. (*a*) Birefringence of synaptic lamellæ. Frog, thoraco-humeral muscle. Treated with 10 per cent $Pb(NO_3)_2$ for 10 min. Frozen section (× 700). Arrows point to lamellar structures. *M*, myelinated nerve fibres. (*b*) The same microscopical field in the state of compensation. ($\Gamma m\mu = 22$)

In the course of further investigations it was found that this vitally persisting organization could be stabilized by immersing the fresh muscles in a 5–10 per cent solution of lead nitrate for 10–15 min. (This treatment with lead ions had been employed by us in previous experiments[2] for demonstrating —SH groups. The stabilizing action of lead ions might similarly be explained with a mercaptide linkage, so that —SH groups of neighbouring polypeptide chains are linked together with Pb^{++} ions, thus giving stability to the micellar structure.)

Immediately after treatment with lead nitrate, sections were cut with a freezing microtome, washed twice in distilled water and examined under the polarization microscope. Positive birefringence of the synaptical lamellæ with respect to their optical (longitudinal) axes was observed in these sections; the birefringence of muscle fibres, as well as that of collagenous tissue and myelin sheaths, remained unaltered (Fig. 1). Extraction of lipids by floating the sections in warm acetone for 4 hr. or embedding the lead-treated material in paraffin resulted in a 40 per cent increase of birefringence as measured with Senarmont's mica-plate compensator.

The positive birefringence indicates that every single lamella is built up of rod-shaped micelles having an orientation parallel to the axis of the lamella. The fact that extraction of lipids increases birefringence shows that these micellar rodlets consist of proteins, and, furthermore, that the orientation of the lipids differs from that of the protein micelles. The relatively small increase of birefringence (only 40 per cent) caused by extraction suggests that lipids are organized to a lesser degree than proteins.

Fig. 2 illustrates the degree of birefringence of a single lamella as a function of the refraction index of the embedding medium (lipids extracted). The

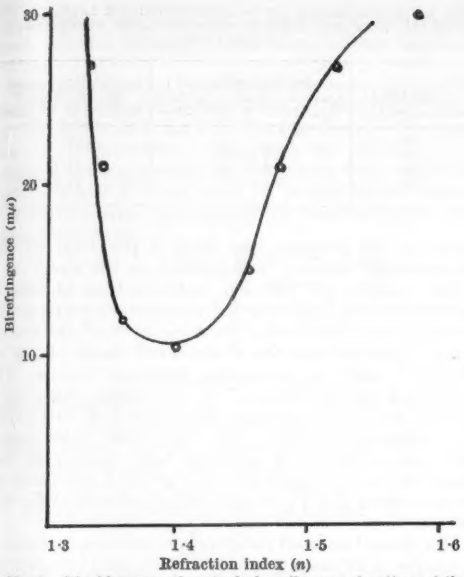

Fig. 2. Birefringence of a single lamella as a function of the refraction index of the embedding medium

curve does not decline to zero even at the point of its minimum ($n = 1{\cdot}4$). This rest (crystalline) birefringence indicates that the intralamellar micelles themselves are birefringent, that is to say, the polypeptide chains are oriented inside the micelles. The positiveness of the rest birefringence proves the longitudinal orientation of these chains.

According to our investigations, the highly specialized function of the postsynaptic membrane is rendered possible by a highly organized and at the same time very labile structure. Details concerning the submicroscopical pattern of other synaptical structures will be reported later.

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5-Hydroxytryptamine in Blood during Experimental Traumatic Shock in Rats

It has been shown that the level of histamine in blood increases very much during experimental traumatic shock in rats[1]. Evidence has been presented that body 5-hydroxytryptamine (5-HT) might be influenced by the same factors as histamine[2–4]. We therefore investigated blood-levels of 5-hydroxytryptamine during experimental traumatic shock in rats.

Adrenalectomized white rats were subject to shock by limb ischæmia[5]. Rubber tourniquets were applied on both hind legs for 2 hr.; 45 min. later, when the signs of the shock became evident, the animals were killed and blood samples taken. 5-Hydroxytryptamine was extracted with 4 volumes of acetone for 24 hr. and determined on Vane's isolated rat fundus preparation[6]. The results are shown in Table 1. During shock, the blood-level of 5-hydroxytryptamine was found to be approximately four times as great as the normal value ($P < 0{\cdot}001$). Table 1 shows that the level of 5-hydroxytryptamine in the blood decreased in adrenalectomized control animals as compared with that found in normal rats (for which the values obtained were in good agreement with those found by other authors[7]). This indicates that adrenalectomy might influence blood-levels of 5-hydroxytryptamine.

Table 1. 5-Hydroxytryptamine in Rat Blood

	(1) Controls, normal	(2) Controls, adrenal-ectomized	(3) Traumatic shock adrenal-ectomized	*P*
5-HT (μgm./ml.) (mean ± *S.E.* of the mean)	0·276 ± 0·050	0·095 ± 0·010	0·371 ± 0·052	3 : 2 <0·001

The mechanism by which the level of 5-hydroxytryptamine in blood is raised during traumatic experimental shock is unknown. It is possible that some toxin from the ischæmic limb releases this substance. Hæmoconcentration, which ensues during shock with the increased number of blood platelets, could not significantly influence blood-levels of 5-hydroxytryptamine, since, while hæmoconcentration amounted to 130 per cent, the level of 5-hydroxytryptamine in the blood was increased to 400 per cent.

As to the significance of the elevation of blood-level of 5-hydroxytryptamine during experimental traumatic shock, it should be pointed out that this substance was found to be released from blood platelets during anaphylactic shock in rabbits[8], but the signs of this shock in the guinea pig were found not to be causally related to 5-hydroxytryptamine[9]. On the other hand, evidence exists that the anaphylactoid reaction in rats is mediated chiefly through the release of 5-hydroxytryptamine[10,11]. It is not possible as yet to say whether the increase of 5-hydroxytryptamine in the blood which occurs during experimental traumatic shock is causally related to the appearance of signs of this condition. However, it should be noted in this connexion that 5-hydroxytryptamine was found, like histamine, to increase the permeability of blood vessels[3,4].

The mechanism and the possible causal relation between the elevation of 5-hydroxytryptamine in the blood and the signs of the experimental traumatic shock are being investigated further.

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Incorporation of 5-Iodouracil labelled with Iodine-131 into the Deoxyribonucleic Acid of Human Leukæmic Leucocytes following *in vivo* Administration of 5-Iododeoxyuridine labelled with Iodine-131

The extensive introduction under cell culture conditions of 5-bromodeoxyuridine and 5-iododeoxyuridine into the deoxyribonucleic acid of a cell strain derived from a human tumour was recently reported[1,2]. Prusoff *et al.* and Mathias and Fischer[3–6] have recently reported *in vitro* and *in vivo* animal studies with 5-iododeoxyuridine. *In vivo* studies in this laboratory have been oriented towards possible uses of labelled 5-iododeoxyuridine in the investigation of nucleic acid metabolism of human leukæmic leucocytes as well as other tissues in which cell proliferation is extensive.

Experiments in animals showed that the labelled 5-iododeoxyuridine was incorporated to the extent of 0·5–1 per cent into the deoxyribonucleic acid fraction of intestine and tumour of rats bearing 7-day *H.S.* No. 1 and *H.Ep.* No. 3 human tumour transplants[7]. Toxic effects were not observed following daily administration of 5-iododeoxyuridine at 33 mgm./kgm. for one week in the mouse and two doses at 6·6 mgm./kgm. to a dog on successive days.

The incorporation of the labelled iodouracil moiety into the deoxyribonucleic acid of circulating leucocytes was measured following intravenous administration of 0·62 mc. labelled 5-iododeoxyuridine (9·6 mgm.) to a 44-year old, 85-kgm. woman with previously untreated chronic granulocytic leukæmia and enlarged spleen and liver (Table 1). Incorporation of iodine-131 into the thyroid was prevented by repeated administration of potassium iodide. Sodium nucleates were extracted from the washed white cells in hot salt solution. Following dialysis of this extract, the ribonucleic acid was hydrolysed in alkaline solution and the ribonucleotides, after neutralization, removed by a second dialysis. The deoxyribonucleic acid was hydrolysed to deoxyribonucleosides by treating an aliquot of the resulting solution with deoxyribonuclease (0·0025*M* magnesium sulphate, *p*H 7) at 37° for 5 hr. followed by addition of snake venom phosphoesterases for an additional 5 hr.[8]. After coagulation of protein at 100°, aliquots of the supernatant were subjected to descending paper chromatography in *iso*propanol/hydrochloric acid/water. Labelled 5-iododeoxyuridine and thymidine were eluted. The activity of the former was measured using a scintillation counter and the μmol. of the latter determined by ultra-violet absorption. The incorporation of labelled 5-iododeoxyuridine into the deoxyribonucleic acid is expressed in column 2 as counts per min. (c.p.m.) labelled 5-iododeoxyuridine/μmol. thymidine. The possibility of contamination was eliminated by paper chromatographic studies which showed that the labelled 5-iododeoxyuridine could be detected only after treatment with deoxyribonuclease and snake venom phosphoesterases. The major portion of the administered radioactivity was eliminated in the urine (column 3). Essentially all the radioactivity was present in the urine as labelled iodide ion with the exception of the initial 2-hr. collection, which contained 8 per cent of the total administered radioactivity. In this sample 11 per cent of the activity was present as labelled 5-iododeoxyuridine. In a previous experiment in the human, less than 1 per cent of the administered activity was present in the stool.

Table 1. Incorporation of 5-Iododeoxyuridine labelled with Iodine-131 into the Deoxyribonucleic Acid of Circulating White Cells

Hours after administration	Deoxyribonucleic acid: c.p.m. 5-iododeoxyuridine/micromole of thymidine	Cumulative percentage excretion of iodine-131 in urine
24		53
48	$2·7 \times 10^3$	67
120	$4·9 \times 10^3$	72·1
168	$2·0 \times 10^3$	72·4

The results at 120 hr. indicate that at least 1 per cent of the administered radioactivity was incorporated into the deoxyribonucleic acid of the leucocytes. This is based on a white cell count of $2·5 \times 10^5$/mm.^{3} and the following assumed values: 6 l. estimated blood volume, 7×10^{-9} mgm. deoxyribonucleic acid/cell—corresponding to $0·6 \times 10^{-8}$ μmol. thymidine/cell. Since the circulating cells represent only a portion of the total body leucocytes, the extent of incorporation into the latter is estimated at several times the 1 per cent value calculated for the former.

Hamilton has used radiocarbon-labelled precursors of nucleic acid bases to study nucleic acid turnover in human leukæmic cells[9,10]. Since labelled 5-iododeoxyuridine is an analogue of thymidine, its incorporation into leucocyte deoxyribonucleic acid as a function of time after administration may also yield useful information concerning nucleic acid metabolism. The short half-life (8·0 days) of iodine-131 relative to radiocarbon and tritium may be of advantage in certain studies in the human.

In an attempt to evaluate differential energy absorption in white blood cells as compared with whole blood, the iodine-131 activity of plasma, red blood cells and white blood cells was determined over a 12-day period. The concentration of iodine-131 in white blood cells was greater than that in red blood cells and plasma during this period with a maximum factor of 40 on the sixth day. By integrating the areas under the curves for each of these fractions, estimates of radiation delivered to packed white blood cells and whole blood were made. The β-radiation for packed white blood cells and whole blood was 0·94 and 0·23 rad/mc. of dose respectively. The γ-radiation affecting the whole body was calculated from the body retention curve, assuming that 40 per cent of the γ-ray energy was absorbed in the body. This added 0·35 rad/mc. In this way, the total radiation absorbed in packed white blood cells was estimated to be 2·2 times that of whole blood. In so far as radiation conditions for packed white blood cells can simulate that for leukæmic infiltrates, this would be a favourable factor in consideration of possible radiotherapeutic applications[11].

Counting at skin contact was carried out with a scintillation crystal at intervals for two weeks after administration of 5-iododeoxyuridine. The splenic area yielded a higher count than any other. At 6 days the difference between spleen and mid-thigh area was maximal and the organ-thigh ratio was 27 for spleen, 11 for liver, and 6 for cæcum and urinary bladder.

The dependence of the extent of incorporation of iodine-131 into the deoxyribonucleic acid of rapidly proliferating tissues on the mode of 5-iododeoxyuridine administration is under investigation. Measurement of differential energy absorption in a number of tissues

will facilitate appraisal of any potential radiotherapeutic use for labelled 5-iododeoxyuridine administration.

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Temperature Control of Physiological Dwarfing in Peach Seedlings

One of the key problems in germination physiology is the mechanism of embryo 'dormancy' or rest. One aspect of this problem is the physiological dwarfing which has been reported to occur in the seedlings of woody plants, especially of such commercial fruits as peaches. These seeds normally require an extended period of low-temperature after-ripening to permit germination. However, if the seed-coat is removed from non-after-ripened seeds germination occurs, but the shoots of such plants are abnormal with telescoped internodes and leaves which may be deformed or sometimes reduced to a rosette of white, scale-like appendages[1,2]; the exact symptoms are a varietal characteristic[2]. Low-temperature after-ripening of the seed or seedling is commonly reported to be essential to produce a normal plant.

The symptoms of physiological dwarfing in seedlings are similar to those shown by buds of trees forced after insufficient chilling. Several authors have therefore suggested[1,4,5] that the mechanisms of physiological dwarfing and the rest period in buds may be the same. In view of this similarity, and a growing body of evidence that the rest period is the result of the action of chemical inhibitors[6], several anomalous features of this phenomenon should be recognized. In the first place, dwarf seedlings have been reported to remain growing but dwarfed for as long as ten years[3], a period inconsistent with the known stability of compounds in biological systems. Secondly, the dwarfing symptoms are restricted to the leaves and stem produced directly by the epicotyl apex[1,3]; branches formed are always normal. Such precise anatomical localization is difficult to correlate with an inhibitor molecule of sufficiently small size to be chromatographed and detected in an auxin analysis system.

Table 1. Effect of Germination Temperature on the Development of Dwarfing Symptoms in Elberta Peach Seedlings from Non-after-ripened Seeds

	Germination in light			Germination in dark		
	19° C.	23° C.	27° C.	19° C.	23° C.	27° C.
Average height (cm.)	17·4	16·8	15·7	17·7	17·7	15·0
Average number of nodes per plant	18·5	16·8	15·2	17·4	18·9	14·6
Average number of nodes with abnormal leaves	0	0·8	5·5	0·7	1·7	7·1
Relative severity of dwarfing (0–4)	0	0·3	2·2	0·6	0·9	3·7

Recently we have discovered a third anomaly: physiological dwarfing is not an obligatory state in the development of a seedling from a non-after-ripened seed. Such seedlings may be either dwarf or normal; the expression, or failure thereof, of the dwarfing character is a function of the temperature to which the seed is exposed during the first few days of germination. The temperature-range for this effect is extremely small, approximately 4° C., and lies within the range of 'normal' germination temperatures.

Results of a typical experiment showing this temperature effect are presented in Table 1. In this experiment, seeds of *Prunus persica* Sieb. and Zucc. var. Elberta were sterilized by immersion for 10 min. in a 5 per cent suspension of 'Pittchlor' (Columbia, Southern Chemical Corp.), rinsed, and allowed to imbibe in the dark at 25° C. for 24 hr. The seed-coat and endosperm for about one-third the length of the seed at the hypocotyl end were excised aseptically and the seeds germinated, approximately ten in a 15-cm. Petri dish. Three germination temperatures were used. At each temperature one dish was exposed to continuous illumination 70 cm. from 40-watt standard cool white fluorescent tubes on 2-in. centres with 20 per cent added incandescent wattage. A dark control at each temperature was obtained by wrapping a dish in black polyethylene. To distinguish between light and temperature effects, the dishes rested in stainless steel trays immersed in constant-temperature water baths adjusted to give the desired seed temperature. This was necessary since high-intensity fluorescent lights produce sufficient heat to raise the level at the germinating surface considerably above ambient temperature unless special precautions are taken. After germination for seven days, the seedlings were planted in coarse white sand, watered daily with a mineral nutrient solution, and grown under the fluorescent lights described above on a 16-hr. day with day temperature of 27° C. and night temperature of 22° C. After a growing period of 5 weeks the height of the plants was measured and the total number of nodes counted, including nodes with scale leaves (germination in peach is hypogeal). In Elberta peaches grown under these conditions, dwarfing is primarily shown by distorted leaves which are abnormally wide but short and twisted as a result of the failure of midrib elongation. Severity of dwarfing was scored in two

ways: (1) on an arbitrary scale of 0–4 based on a visual estimate of degree of abnormality; (2) by counting the number of nodes with abnormal leaves.

From the results, it is clear that there is little if any difference between seedlings germinated in the light and those germinated in the dark. Germination at the higher temperatures results in a slight reduction in height of plant and number of nodes. The major effect of temperature is on the development of dwarfing symptoms. At 19° C. all plants were essentially normal; at 27° C. they were all severely dwarfed. At 23° C. only a few plants showed dwarfing symptoms and few of these were severe. Thus the effective range for the development of dwarfing symptoms is approximately 4 deg. C., between 23° and 27° C. The effective time lies within approximately the first week of germination, since subsequent growth of all plants was at the same temperature.

Thus far we have observed this response in only one peach variety; it is essential to determine whether it is of general occurrence. If dwarfing is temperature-controlled in a number of varieties and species, then immediate practical applications to fruit tree breeding will be possible. The major importance of this observation, however, may lie in suggesting possible new explanations for the mechanism of the rest period. We are therefore continuing the investigation.

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PATHOLOGY

Changes in Specificity and Electrophoretic Mobility of the Precipitating Antibodies present in the Serum of Cattle recovering from Foot-and-Mouth Disease

Graves[1] has shown by agar gel diffusion tests that quantitative differences exist among three different field samples (Channel Island, Ven. 1 and *M.*11) belonging to the same immunological type (Vallée O) of foot-and-mouth disease virus. He noticed, however, that these sub-type differences could not be clearly shown when early convalescent cattle serum (7–14 days) was used for the tests. This was due to the almost equal reaction of the immune serum with the heterologous as well as the homologous virus sub-type. During an examination of the nature of this cross-reaction by immunoelectrophoresis some interesting observations have been made regarding the mobility of the antibodies present in the sera during early convalescence. Cattle sera collected 7, 14 and 21 days after infection with the Channel Island sub-type of the virus were separated electrophoretically in agar by the method of Williams and Grabar[2]. The separated serum proteins were then allowed to diffuse towards the homologous Channel Island virus on one side or the heterologous sub-type *M.*11 on the other. The 7-day convalescent serum gave a precipitin line with both sub-types of the virus whereas the 14 and 21 days sera precipitated only with the homologous Channel Island virus (Fig. 1). In addition to this cross-reaction it was observed that the precipitin lines formed by the 14 and 21 days sera were longer and nearer to the cathode than those formed by the 7 day serum.

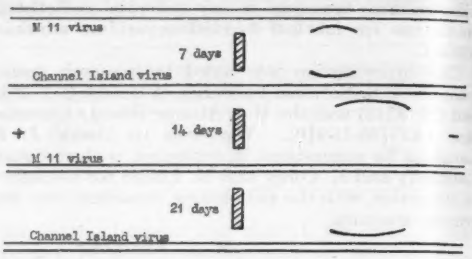

Fig. 1. Immunoelectrophoresis of convalescent sera taken 7, 14 and 21 days after infection with the Channel Island strain of the virus

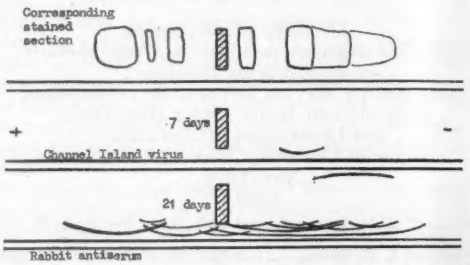

Fig. 2. Immunoelectrophoresis of convalescent sera from cattle after infection with the Channel Island strain of the virus. The positions of the precipitin bands obtained by diffusing the separated proteins towards the homologous virus and rabbit antiserum are compared

In view of this marked difference in mobility it appeared of interest to examine further the nature of the antibodies. The 7 and 21 days sera, following electrophoresis in agar, were allowed to diffuse towards rabbit anti-bovine serum and the positions of the precipitin bands compared with those obtained by diffusion towards the homologous virus. The results of such an experiment are shown in Fig. 2. It appears, on this evidence, that the precipitating antibody present in the 7-day convalescent serum corresponds to a β-type globulin. The precipitin bands obtained when the 7 and 21 days sera are allowed to diffuse from adjacent cups towards the homologous virus do not cross, however, indicating that the antibodies are identical. In addition, a mixture of the 7 and 21 day sera gives one continuous line when examined by immunoelectrophoresis. It may be, therefore, that the antibodies present in both the 7 and 21 days sera are γ-globulins of different electrophoretic mobilities.

Some further evidence has been obtained of the different physical properties of the antibodies present in the two sera. When the 7-days serum, dialysed against 0·01 *M* phosphate *p*H 7·6, is subjected to chromatography on *DEAE*-cellulose in equilibrium with the same buffer solution no virus-neutralizing activity is found in the eluate although complete recovery of this activity can be achieved by elution with 0·05 *M* phosphate *p*H 4·7. On the other hand,

when the 21-days serum is subjected to chromatography under the same conditions a considerable proportion (more than 50 per cent) of the neutralizing activity initially present in the serum is obtained in the 0·01 *M* phosphate fraction. It is evident that differences exist in the antibodies present in the two sera and these differences are being examined in more detail.

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Response of Newly Born Mice to a Chemical Carcinogen

Many recent reports record that certain leukæmias and malignant lymphomas can be transmitted to newly born mice using cell-free extracts of tumour tissues[1,2]. Under certain conditions, cell-free extracts of leukæmic organs also produced parotid gland tumours, sarcomas and other neoplasms[3–5]. Tissue culture extracts and extracts of tumour tissues[6–10] have given rise to leukæmias and other tumours in rats and hamsters, following injection into the newly born animal.

Since all these studies have made use of newly born animals, it seemed to us that general studies of the pathological responses of the newly born animals were of considerable importance. We have investigated the effects of certain chemical carcinogens in this same biological system. This communication reports the effects of small doses of the purified carcinogenic hydrocarbon, 9,10-dimethyl-1,2-benzanthracene, administered subcutaneously as a colloidal suspension to newly born Swiss mice. The Swiss mice were originally obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and have been bred at random in this laboratory for the past nine years. The incidence of tumours occurring in these animals has been carefully followed in many untreated controls during this time; malignant lymphomas of different types have been found in 4 per cent of animals of more than one year of age but have not been seen prior to this time. A slightly higher incidence of malignant lymphomas (6 per cent) has been noted following a single dose of 9,10-dimethyl-1,2-benzanthracene given by various routes of administration to animals 8–10 weeks old. The earliest lymphomas seen in these animals occurred at 42 weeks of age.

In the present experiment 25 newly born Swiss male and female mice, less than 12 hr. of age, were given a single subcutaneous injection of 30 μgm. 9,10-dimethyl-1,2-benzanthracene as a colloidal suspension. The suspension was prepared by the rapid addition of 9,10-dimethyl-1,2-benzanthracene, dissolved in acetone to 1 per cent aqueous gelatin, kept at 56° C. in a water-bath. The acetone was then removed by slowly bubbling nitrogen through the suspension. Newly born mice were injected subcutaneously in the interscapular region (tuberculin syringe, 26 gauge needle) with 0·015 ml. of the suspension, containing 30 μgm. 9,10-dimethyl-1,2-benzanthracene. The first animal was killed when moribund at the eleventh week from birth and showed a marked enlargement of the thymus which occupied a large part of the chest cavity, compressing the lungs. The spleen and peripheral lymph nodes appeared normal in size and colour. Three more animals were killed when moribund at the thirteenth week; one showed a marked enlargement of the thymus, the lymph nodes and spleen; the other two had an enlarged thymus and multiple lung adenomas less than 1 mm. diameter. Four additional mice, killed at the sixteenth, eighteenth and twenty-fourth weeks, all had enlargement of the thymus, lymph nodes, spleen and multiple lung adenomas. Histologically, these tumours are malignant lymphomas of the stem-cell type with early lymphocytic differentiation. Microscopic infiltrations of lungs, kidneys, skeletal muscles, spleen, lymph nodes, salivary glands and bone marrow were found; but not all these organs were involved in every animal. Thus, eight mice of a group of 25 have developed lymphomas in the short period of 11–24 weeks, at an average age of 15·3 weeks following injection (an incidence of 32 per cent). Control mice were given injections of a similar amount of colloid only; all survive and no tumours have occurred so far.

The finding that polycyclic hydrocarbon carcinogens can give rise to or enhance leukæmia has been reported many times using different strains of mice[11–14]. The present study differs from prior investigations in that only a single, low dose of carcinogen has given rise to a high incidence of malignant lymphoma rapidly in a relatively insusceptible mouse. It is emphasized, from this finding, that the development of this disease is dependent, to a great extent, upon factors inherent to the animals at this stage of development. The character of the response encountered in newly born mice with cell-free filtrates should be viewed not only in terms of virus concepts but also as a response of a sensitive biological system to carcinogenesis.

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Complement Fixation with Tissue Culture and Liver Antigens of Mouse Hepatitis

Host-specific viruses associated with hepatitis of duck[1], dog[2], mouse[3–6], and man[7] have been described. The agents of duck[8], dog[9] and mouse[10,11] hepatitis have been propagated in tissue culture. Complement-fixing antigens have been prepared from infected livers of mouse[12] and dog[13]. However, studies on propagation and serology of human hepatitis viruses have as yet yielded equivocal results[14]. By means of virus-neutralization tests in mice, Gledhill *et al.*[15] noted an antigenic relationship among strains of murine hepatitis virus. They stated that *JHM*, Nelson, and *MHV* strains probably form a related group of viruses.

This report describes a complement-fixation test with liver antigens in which a relationship was noted between Gledhill's[16] *MHV*-1 and Nelson's[17] *PRI* mouse hepatitis strains. In addition, complement-fixing antigen was demonstrated in serially passaged tissue cultures of *MHV*-1. Tissue cultures showed no definite cytopathogenic effect but were infective for newly born mice.

The complement-fixing procedure used was essentially that described by Bengtson[18]: 0·2 ml. quantities each of antigen dilutions, serum (inactivated as a 1 : 10 dilution in saline at 61° C. for 20 min.), and complement (2 units) were used; after incubation overnight at 4° C., the hæmolytic system was added; complement fixation was determined visually after subsequent incubation at 37° C. for 30 min.

Complement-fixing antigens were prepared from liver homogenates of suckling mice infected with *MHV*-1 and of weanling mice (8–10 gm.) infected with *PRI* virus. Whole cultures of serially passaged *MHV*-1 in mouse kidney explants[11] were also used as complement-fixing antigens. Later experiments indicated that nutrient fluids alone contained this complement-fixing antigen. Control antigens were prepared from liver homogenates of normal weanling and suckling mice and from uninoculated tissue cultures. Each antigen preparation was centrifuged at 40,000*g* for 1 hr. at 4° C. to eliminate sedimentable non-specific complement-fixing factors which occurred occasionally with some pools of control material centrifuged at lower speeds.

Tissue culture and liver preparations containing *MHV*-1 were assayed for infectivity in newly born Swiss mice[11]. Titres of 8 different *MHV*-1 liver pools had *ID*50 end-points of $10^{-3\cdot0}$ to $10^{-3\cdot6}$/0·05 ml. of inoculum. Ten serially passaged tissue cultures of *MHV*-1 yielded ID50 end-points from $10^{-4\cdot1}$ to $10^{-5\cdot2}$ (Table 2). The *PRI* liver homogenates had *ID*50 end-points from $10^{-3\cdot2}$ to $10^{-3\cdot6}$/0·1 ml. inoculum when assayed in weanling Swiss mice (8–10 gm.).

Table 2. Comparison of Infectivity and Complement-Fixation Titres of Serially Passaged Tissue Cultures of *MHV*-1

Passage No.	Dilution of original virus	Infectivity titre* −log *ID*50		Complement-fixing titre reciprocal of antigen dilution			
		0 hr.	96 hr.	0	2	4	8
1	10^{1}	2·4	4·8	*NT*†	*NT*	*NT*	*NT*
2	10^{3}	3·0	4·6	*NT*	*NT*	*NT*	*NT*
3	10^{4}	2·5	4·8	++++	+++	–	–
4	10^{5}	3·8	5·2	++++	++++	–	–
5	10^{7}	1·9	4·4	+	–	–	–
6	10^{8}	*NT*	4·7	++++	+++	–	–
7	10^{9}	3·7	4·1	++++	++++	++++	–
8	10^{10}	3·5	4·8	++++	–	–	–
9	10^{11}	3·6	4·2	*NT*	++++	++++	–
10	10^{12}	2·7	4·3	++++	++++	–	–

* See ref. 11. † Not tested.

Results of box titration tests showed cross complement-fixing reactions between *MHV*-1 and *PRI* virus (Table 1). Normal liver antigen did not fix complement in the lowest dilutions of serum and antigen tested. However, 1 out of 10 supposedly 'normal' serum pools fixed complement in the presence of *MHV*-1 and of *PRI* liver preparations. As viral agents causing hepatitis in mice have appeared 'spontaneously' during the course of studies on enteric disease of the suckling mouse[6], human viral hepatitis[3,19] and mouse leukæmia[5,17], it is possible that some donors to this positive 'normal' serum pool had in fact been infected with a murine hepatitis virus related to *MHV*-1 or *PRI*.

Antigens processed from *MHV*-1-infected, kidney explant tissue cultures fixed complement in the presence of *MHV*-1 immune serum (Table 2). All serum pools were diluted 1 : 10 in saline and inactivated (61° C. for 20 min.) prior to use. Control serums and antigens were negative in this system. It appears that complement-fixing titres obtained with tissue-culture antigens were somewhat lower than those obtained with liver antigens. Of 18 additional mouse-kidney tissue-culture antigens tested, infectivity titres ranged from *ID*50 $10^{-1\cdot9}$ to $10^{-5\cdot2}$ and complement-fixing titres from 1 : 0 to 1 : 8. A relation-

Table 1. Cross-Reactions between Complement-Fixing Antigens of *MHV*-1 and *PRI* Mouse Hepatitis Viruses

			Liver antigen – reciprocal of dilution									
			MHV-1					*PRI*				
			4	8	12	16	20	4	8	12	16	20
Immune serum – reciprocal of dilution	*MHV*-1	10	++++	++++	++++	++++	+	++++	++++	++++	++	–
		20	++++	++++	+++	++	–	++++	++++	++++	–	–
		40	++++	++++	++	–	–	++++	++++	+++	–	–
		60	++++	++	–	–	–	++++	++++	+	–	–
		80	++++	–	–	–	–	++++	++++	–	–	–
	PRI	10	++++	+++	–	–	–	++++	++++	+	–	–
		20	+++	–	–	–	–	++++	+++	–	–	–
		40	++	–	–	–	–	++++	++	–	–	–
		60	+	–	–	–	–	++++	+	–	–	–
		80	–	–	–	–	–	++++	+	–	–	–

ship was not noted between complement-fixing and infectivity titres in these tests.

A relationship was demonstrated between the *MHV*-1 and *PRI* murine hepatitis strains by complement-fixation tests. Specific antigen was obtained from serially passaged, *MHV*-1-infected, mouse-kidney tissue cultures which fixed complement in the presence of immune serum prepared in mice with extracts of *MHV*-1-infected liver in adjuvant.

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Incorporation of Thymidine labelled with Tritium by Circulating Cells of Infectious Mononucleosis

CONSIDERABLE uncertainty exists about the origin and the identity of the cells of infectious mononucleosis. They have been described as atypical lymphocytes, as monocytes, as reticular-endothelial cells developing towards a lymphomonocyte or even a plasma cell. Most hæmatologists[1,2], however, regard them as mature cells, even though they do vary greatly in size and shape.

Useful information on this subject could certainly be obtained by a functional study of these cells. Autoradiography provides a powerful tool for investigation at a cellular level. Moreover, the availability of compounds labelled with tritium has greatly improved the resolution of this method. Thymidine-^{3}H is known to be a specific precursor of deoxyribonucleic acid; therefore, its incorporation into cell deoxyribonucleic acid is evidence of active deoxyribonucleic acid synthesis, possibly preparatory to later mitosis.

Incorporation of thymidine-^{3}H has been investigated in the peripheral blood of three cases of infectious mononucleosis and of two normal subjects. In infectious mononucleosis incorporation has been followed during the various stages of the disease up to complete recovery. All cases showed a complete clinical picture, a characteristic hæmatological pattern and a highly positive Paul–Bunnel test.

Blood was withdrawn from the antecubital vein and 2 ml. of a 5 per cent dextran solution (molecular weight 150,000) was added to 8 ml. of citrated blood. After 20 min. the supernatant was pipetted into a test-tube; the white cells were washed twice in normal saline with low-speed centrifugation and then resuspended in Eagle's medium. This procedure was carried out under sterile conditions. Thymidine-^{3}H with a specific activity of 390 mc./m*M* was then added to a final dilution of 5 μc./ml. Incubations were in a water-bath at 37° C.; samples of cells were withdrawn after 2, 4 and 6 hr. and smears were made on gelatin-coated slides. The smears were fixed in Carnoy's solution, and, after several washings in distilled water, stripping films (Kodak *AR*-10) were placed over the slides according to the method described by Pelc[3]. The films were developed after ten days exposure and smears stained through the stripping film with May–Grünwald–Giemsa. For each slide 4,000 cells were counted to determine the amount of labelling.

The percentage of labelled cells detected in normal individuals corresponded strictly to that observed by Bond *et al.*[4], that is, 0·06 per cent referred to the number of total white cells; labelled cells were all large lymphocytes or monocytes. In cells from patients affected by infectious mononucleosis considerably higher values were observed (Table 1); in the first stage of the disease, for example, the mean percentage of labelled cells, after 2 hr. incubation, was 4·44, that is, more than thirty times the value for normal blood. This percentage decreased progressively during the course of the disease and, with the complete recovery, reached the normal value. In the first stage of the disease only the percentage of labelled cells increased linearly with the time of incubation, from 4·44 (second hour) to 10·5 per cent (sixth hour). This linearity was no longer detectable in the later stages.

Table 1. PERCENTAGE OF LABELLED LYMPHOMONOCYTES AT DIFFERENT STAGES OF THE DISEASE (AFTER 2-HR. INCUBATION)

Stage	Cases			Mean value	$\sigma \pm$
	1	2	3		
1	3·8	4·3	5·2	4·44	0·57
2	1·1	2·1	1·4	1·53	0·41
3	0·30	0·30	0·15	0·25	0·12
4	0·066	0·075	0·048	0·063	0·011

Stages: 1, Acute phase of the disease; 2, early improvement; 3, full improvement; 4, complete recovery.

Our results clearly show that in infectious mononucleosis the cells of the peripheral blood have a greater tendency to synthesize deoxyribonucleic acid than normal lymphomonocytes. This synthesis may be preparatory to subsequent mitosis, though this cannot be considered as direct evidence of mitosis. No mitotic figures were observed in any of the slides we examined. Mitosis in the peripheral blood of infectious mononucleosis patients has been only occasionally reported[5–7].

The incorporation of deoxyribonucleic acid precursors by bone marrow cells has been used to calculate the mitotic cycle of these cells[5]; in our case, however, the degree of incorporation during the period of incubation does not permit such a study because it is not certain that all mononuclear cells of the peripheral blood will undergo mitosis; moreover, as demonstrated by Painter[9], the radiobiological effect of thymidine-^{3}H incorporated in the chromosomes may, in *in vitro* experiments, affect the proliferative activity of the cells.

The most important fact shown by this investigation is that the cells of infectious mononucleosis behave in a completely different way from the lymphomonocytes of normal peripheral blood with respect to deoxyribonucleic acid synthesis. It is therefore reasonable to consider them as totipotent cells of mesenchymal origin, which actively proliferate in the organs and easily enter the peripheral blood. As an alternative hypothesis one might suggest that the increased synthesis of deoxyribonucleic acid may indicate virus synthesis within the cells.

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BACTERIOLOGY

Measurement of a Staphylococcal Antibody in Human Serum by a Mouse Protection Test

The need for a better understanding of immune phenomena related to staphylococcal infections has been repeatedly emphasized[1]. In so far as humoral antibodies are concerned, a substantial amount of work has been done only with staphylococcal antitoxins. However, as pointed out by Rountree and Barbour[2], this approach has not appeared to be rewarding. It has also been suggested that anticoagulase may be an important antibody[3]; but some doubt exists as to the significance of coagulase in staphylococcal infections[4,5]. Although a promising serological procedure was developed by Rountree and Barbour[2], this method was not applied in a study of infections.

It has been postulated that one of the most significant tests of the importance of antibody is the demonstration of its protective activity in experimentally infected animals[6]. Therefore, the application of the mouse protection test described in this communication may provide a useful new approach for the study of staphylococcal immunity. In addition, this procedure not only provides acceptable sensitivity but can also be used to measure the staphylococcal antibody content of a given serum.

Since the mechanism of action is at present unknown for this antibody, it is described functionally as 'mouse protective antibody'. The specificity of mouse protective antibody has been established by absorption procedures, similar to those used for determining antibody specificity in human gamma globulin[7], and will be reported elsewhere. Its 'classic' antibody nature is also supported by the fact that mouse protective antibody potency was unaltered by exposure to 60° C. as long as 4 hr.

In performing a mouse protective antibody assay, 18–22 gm. albino Webster mice, either sex, are injected intraperitoneally with 10–20 *LD*50 of the Smith strain of *Staphylococcus aureus*. This inoculum, consisting of about 10^4 cells, is derived from a 12–18 hr. broth culture and is suspended in 5 per cent hog gastric mucin, using 0·5 ml./mouse. As described by Hunt and Moses[8], this dose of Smith staphylococcus will produce an acute systemic infection, lethal to 90–100 per cent of untreated mice within 24 hr. Although most of these studies have involved the Smith strain, comparable activities of given serums have been obtained with other staphylococcal strains.

Within 30 min. of challenge, groups of mice are injected intraperitoneally with 0·25 ml. each of varying saline dilutions of the serum to be titrated. A preliminary test is performed first with 40 mice: 10 untreated controls and three groups of 10, using serum dilutions of 1 : 10, 1 : 100 and 1 : 1,000. The five-day survival values obtained from this test are used as a guide for setting up a definitive titration. In the second assay, groups of 15–20 mice and twofold serum dilutions are employed. Usually, four serum dilutions will suffice for obtaining survival data ranging between 0 and 100 per cent, so that a minimum total of 115 mice is required for the preliminary and definitive assays. As indicated, a five-day observation period will provide final survival results, and, in general, no more than 2 ml. of serum are required for the entire assay.

A typical set of results is given in Table 1 for a given serum, illustrating the preliminary and definitive assays and the increasing protective activity of increasing amounts of serum. The survival percentages derived from the definitive assay are employed for the estimation of mouse protective antibody titre, which can be expressed as the 50 per cent protective dilution (*PD*50). A practicable means of determining the *PD*50 with such survival data is the Miller–Tainter method[9]. The *PD*50 derived from the results shown in Table 1 is given at the bottom of the table.

Table 1. Representative Results of Mouse Protection Tests, Preliminary and Definitive, of a Human Serum Specimen

Serum dilution	Survivors/total	Survivors (per cent)
Preliminary:		
None (untreated)	0/10	0
1 : 10	10/10	100
1 : 100	9/10	90
1 : 1,000	1/10	10
Definitive:		
None (untreated)	1/20	5
1 : 100	19/20	95
1 : 200	16/20	80
1 : 400	8/20	40
1 : 800	3/20	15

50 per cent protective dilution, 1 : 350

Analyses of 50 sera from normal adults revealed *PD*50 titres ranging between 1 : 3 and 1 : 2,000. The geometric mean titre of this group was 1 : 192, as calculated by Perkins's method[10]. Limited studies have shown that persons with a chronic staphylococcal infection, furunculosis, had serum *PD*50 titres in the low range : 1 : 10 to 1 : 60. It has also been noted that, out of 54 pairs of maternal and newly born (cord) sera, 41 exhibited comparable mouse protective antibody, but 13 cord sera were substantially lower in mouse protective antibody content than were the respective mothers. This observation suggests, as noted by Rountree and Barbour[2], that in some instances there may be a placental barrier to the transmission of staphylococcal antibody to the fœtus. Studies are in progress to determine the incidence of staphylococcal infection among the newly born and their mothers with respect to their mouse protective antibody titres.

The widespread occurrence of staphylococcal mouse protective antibody in adult human sera indicates that this is a normal antibody, a characterization that was previously applied by me for staphylococcal and other bacterial mouse protective antibody in pooled human gamma globulin[11]. This conclusion has been recently substantiated by Jensen[12], who employed a qualitative agar gel precipitin method for demonstrating staphylococcal antibody in human sera.

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March 26.

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Immunizing Properties of Insoluble Cell Material derived from *Brucella*

An insoluble fraction was obtained by high-speed centrifugation from *Brucella suis*, after disintegration in a sonic vibrator[1]. This fraction could absorb the agglutinating and mouse protecting antibodies from *B. suis* antisera. Since similar absorptions could be carried out, although less effectively, with whole bacteria, the insoluble material was assumed to be situated on the cellular surface and tentatively regarded as part of the cell wall.

The present work was concerned with the immunizing properties of two insoluble fractions derived from *B. abortus* and *B. suis*. The starting material consisted of bacteria killed and dried with acetone, disintegrated in a 9-kc./s. Raytheon magneto-striction oscillator. The sonic extract was centrifuged in a Sorval centrifuge at 10,000 r.p.m. and the sediment freed from intact cells by differential centrifugation. Fraction 1 (*F*1) was thus obtained. Fraction 2 (*F*2) consisted of smaller particles sedimented from the supernatant fluid left from the first centrifugation, after spinning for 30 min. in a Spinco model *L* ultracentrifuge at 40,000 r.p.m. The immunizing activity of these fractions was compared in mice with that of whole cells killed and dried with acetone, following a method previously used in passive protection experiments[2]. 0·01–1 mgm. quantities were injected intra-abdominally into groups of 5–10 mice. At various times after immunization (2–14 weeks) the immune animals were challenged, together with controls, with 30,000 virulent *B. abortus* (strain 2308) cells administered intra-abdominally. 7 days later the mice were killed with ether, the spleens removed, ground with glass sand and plated on trypticase soy agar. Protection indices were calculated as the ratio of the number of *Brucellae* in the spleens of normal mice to that in the spleens of the immune mice. Higher indices were consistently obtained with the insoluble fractions and the small particle fraction (*F*2) appeared to be more effective than *F*1 (Table 1).

Table 1. Immunizing Activity of Whole, Dried *Brucella* Cells and of Insoluble Fractions derived from Them

Immunizing antigen	Protection indices when challenge given at weeks after immunization				
	2	3	6	12	14
B. abortus whole cells	270	—	30	—	8*
B. abortus Fraction 1	541	—	750	—	53*
B. suis whole cells	25	—	—	—	—
B. suis Fraction 1	222	236	—	50	—
B. suis Fraction 2	—	3,535	—	600	—

One immunizing injection was administered. Groups marked with an asterisk received 1 mgm. antigen, the others received 0·1 mgm. —, Not done.

The higher efficiency of the insoluble fractions, as compared with whole bacteria, was evident in many other experiments not reported here.

It appears very likely, in view of their serological properties, that the insoluble fractions are cell-wall fragments of various sizes, as already stated in a previous publication[1]. Chemical and spectrophotometric determinations are in progress to confirm this assumption. Although little studied so far[3], cell-wall material has already been found a good immunizing antigen[4,5], and the present work shows it to be of promise in immunization against *Brucella*. Recent work[6,7] suggests, however, that some of the activity shown by the cell wall may be non-specific. Experiments are under way to determine to what extent the effects described above might be reproduced with non-related cell-wall material.

This is a preliminary report on an investigation carried out by one of us (J. M.) in partial fulfilment of the requirements for the Ph.D. degree of the Hebrew University—Hadassah Medical School.

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BIOLOGY

Atmospheric Pollen in Relation to Land Use

That the present provides the key to the past is a geological commonplace, and considerable attention has been paid accordingly to the interpretation of pollen diagrams in the light of pollen analyses of recent or surface samples[1,2]. Formerly such investigations centred mainly on the composition of a fossil pollen spectrum as a reflex of its contemporary tree flora. To-day interest, more particularly in western Europe and especially in Britain, has widened to include not merely the composition of forests in prehistoric and early historic time but also the extent and character of the vegetation which, as the result of human activity or of other causes, has more and more replaced forest. A consideration of the composition of to-day's pollen rain in relation to present land use has therefore become desirable.

In the course of an atmospheric pollen census begun in 1942, and still continuing[3], pollen spectra have been obtained from some fifteen centres in Great Britain; but most of them are from inland localities in the lowlands and therefore so heavily weighted with local pollen as to be of little use as reflexes of regional (as opposed to strictly local) land use. Two complete and continuous sets of daily analyses have, however, been made for five years (1945–49) in each of two high, remote and virtually treeless localities among the Brecknockshire mountains, namely, one at Storey Arms on the Beacons road (1,400 ft.) between Merthyr Tydfil and Brecon and the other at Grwyne Fawr Reservoir (1,600 ft.) in the heart of the Black Mountains. A summary of the results of the two sets of analyses is set out in Table 1. The figures in the column headed T.T.P. (total tree pollen) indicate the total numbers of the pollen grains usually included by palæopalynologists under the heading of total tree pollen, namely, birch, pine, elm, oak, lime, alder, beech, hornbeam and ash; those in the columns headed N.T.P. (non-tree pollen) include all herbaceous pollens; other arboreal pollens than those under T.T.P., and all damaged and unidentified grains have been omitted, and the relation between N.T.P. and T.T.P. is seen to be much the same at both stations.

The kinds of pollen (listed above) that make up the T.T.P. are produced by broad-leaved woodlands and pine plantations together, while those that form the N.T.P. come very largely from cultivated land, though some (probably a relatively small proportion) come from the large area recognized as rough grazings (here almost all heavily grazed mountain land). The relevant figures for the respective types of land use have been extracted from the Forestry Commissions Census Reports[4,5] allowance being made for small woods and hedgerow trees[6] and the Ministry of Agriculture's Agricultural Statistics 1945–49 [7], respectively, and are set out in Table 2 first for Brecknockshire alone and then (since some of the pollen trapped must have come from outside that county) for Brecknockshire and five adjacent counties together (Cardiganshire being omitted as too remote).

Table 1. Pollen Deposition on Brecknockshire Mountains, 1945–49

(Grains caught and identified on 5 sq. cm. of horizontal surface)

Station	Total tree pollen (T.T.P.)	Non-tree pollen (N.T.P.)	N.T.P. as percentage of T.T.P.
Grwyne Fawr (Black Mountains)	7,594	22,652	297
Storey Arms (Brecon Beacons)	9,046	27,798	310
Two stations together	16,640	50,450	303

In Table 2 the area entered as arable includes rotation grasses: rather more than one-third of this grassland, taken together with the 'permanent grass', was mown in 1947. It will be seen that whereas N.T.P. on the Brecknockshire mountains is about 300 per cent of T.T.P. (Table 1), the corresponding area of cultivation ('crops and grass') is between 420 and 630 per cent, or, say, 500 per cent, while the area of cultivated land and rough grazings together is between 1,120 and 970, or, say, 1,000 per cent, that of pollen productive woodland. Hence the pollen productivity (in T.T.P.) per unit area of woodland, as measured by pollen precipitation in an area partly fringed (at a distance here averaging 5 miles) by woods, must lie between 5/3 and 10/3 times (that is, between 166 and 333 per cent) of the pollen productivity (in N.T.P.) per unit area of non-forest land lying around the same periphery. Whether the lower or the higher figure is more nearly correct must depend on the pollen productivity of the rough grazings which immediately surround both stations and which, to judge from their botanical composition and the heavy grazing to which they are subjected, must in general be low.

The composition of the pollen rain at these two stations has been worked out in some detail. Thus, grass pollen on the Brecknockshire mountains makes up four-fifths of the N.T.P., that is, it is equivalent to say 250 per cent of the T.T.P. Dr. Verona Conway[8], in her studies of Pennine blanket peats at altitudes varying from 1,200 to 2,050 ft., recorded, in her Zone VIII (modern), grass pollen values of 15–300 per cent of T.T.P., and obtained also from surface blocks values up to 300 per cent. Having regard to the much greater disparity at present existing between woods on one hand and crops, grass and rough grazings on the other in Derbyshire (from which county most of the pollen precipitated on the southern Pennines is derived) as compared with South Wales, a much higher grass pollen figure than 300 per cent might be expected in the former area to-day. It seems likely, therefore, that, provided differential pollen destruction during fossilization can be excluded, Dr. Conway's highest figures relate to a time when deforestation was much less widespread than it is now.

Table 2. Land Use in and around Brecknockshire (Acres)

	Woodlands productive of T.T.P.	Crops and grass				Rough grazings	Crops, grass and rough grazings together		Total land area
		Arable including rotation grasses	Permanent grass	Total	As percentage of woodlands		Total	As percentage of woodlands	
Brecknockshire	36,000	69,349	82,011	151,360	420	251,698	403,058	1,120	466,347
Six counties	238,000	646,473	860,078	1,506,551	630	798,260	2,304,811	970	2,751,772

Again, pollen of ribwort plantain (*Plantago lanceolata*, an almost invariable concomitant of agriculture from Neolithic times onwards)[9] plays an important part in analyses of pollen to-day. It is, next to grass and nettle pollen, the largest constituent of the N.T.P. precipitated on the Brecknockshire mountains, giving values (in terms of T.T.P.) of 10·6 per cent at Storey Arms, 13·2 per cent at Grwyne Fawr, and an average of 12·2 per cent for the two taken together. Godwin[10] obtained similar counts of plantain pollen (up to 10 per cent of T.T.P.) from Neolithic or post-Neolithic lake deposits on the edge of the East Anglian Breckland; the same author's analyses of peat datable between the Middle Iron Age and the middle of the Roman period in Somerset[11] gave much higher values (up to 60 per cent of T.T.P.). Since deforestation and the spread of agriculture can scarcely have been more general in Somerset around A.D. 0 that it is in South Wales to-day, the figures last quoted would seem to be due to quite local influences.

It is hoped to publish the results of this research more fully elsewhere. This work received generous aid in the form of Government grants voted by the Royal Society.

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Detachment of Conidia by Cloud Droplets

It has been found by experiment that conidia may be detached from conidiophores by the impact of minute water droplets in a moving mist or cloud. A preliminary report of this is given since it appears to be a mechanism of spore detachment which has not yet been described.

In an initial experiment, an open Petri dish of nutrient agar was held behind a culture of *Verticillium albo-atrum*, and the colony sprayed with water droplets from a simple atomizer so that some of the droplets swept the colony and were impacted on to the surface of the agar in the dish beyond. The dish was then covered with a lid and incubated. When examined, a number of *Verticillium* colonies were found among the numerous common air-borne mould fungi which developed. No *Verticillium* colonies appeared on control plates when a culture was subjected to puffs of air (with no water droplets) from the same atomizer. Spore detachment was therefore concluded to have been due to the impact of the minute water droplets.

Following this observation, pieces of grass culm and cotton thread soaked in nutrient agar were inoculated with dense spore suspensions of mould fungi and incubated at 100 per cent relative humidity. When the conidiophores were developed, the cultures were placed in air streams into which a continuous stream of water droplets was introduced when required. These droplets were in the size-range 8–210μ diameter with a modal diameter of 78μ.

Table 1. Numbers of Spores removed from *Cladosporium* Colonies during 1-min. Exposures to Streams of Air and Air-carrying Cloud Droplets Respectively

Colony	Air speed (m.sec.)	Spores detached by air current	Spores detached by air current plus droplets
1	0·5	350	4,800
2	0·7	200	67,000
3	2	250	51,000
4	5	3,000	18,000
5	10	4,000	32,000

In a series of experiments, each colonized stem or thread was supported across the prongs of a simple wire fork and placed before the intake orifice of a Gregory[1] spore trap. An air stream of known velocity was then drawn over the colony for 1 min., and the spores removed sucked on to the sticky surface of the slide in the trap. After changing the trap slide, the air was turned on again for another minute with cloud droplets being simultaneously directed into the stream flowing over the colony so that the conidiophores were bombarded with the water droplets carried by the moving air.

The results for *Cladosporium* are typical, and show that larger numbers of spores are detached by air currents containing minute water droplets than by air currents of the same velocity without droplets.

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New Members of the British Marine Bottom Fauna

A study is being made of the smaller members of the fauna of marine gravels on the sea-bed in the Plymouth area. One aspect of the results so far obtained is concerned with the array of pygmy species encountered, several of which, as might have been expected, are new to the British fauna.

A bucket of shelly gravel from near the entrance to Plymouth Sound below about 7 fathoms of water (collected by Mr. G. R. Forster during an aqua-lung dive) produced three species of Acochlidiacea, a sub-order of Gastropoda not hitherto recognized as British (though all three have been found near Heligoland). These are *Microhedyle lactea* Hertling, *Hedylopsis suecica* Odhner, and *Philinoglossa helgolandica* Hertling. These animals are shell-less and do not exceed 2 mm. in length, and are scarcely detectable if they cannot be examined alive. A specimen of the small holothurian *Leptosynapta minuta* (Becher) was also obtained from this sample: a species not on the British list, but, as I have since been informed by Dr. D. J. Crisp, discovered at Bangor by Dr. B. Swedmark in 1956.

Chief attention, however, has been given to the Eddystone shell gravel, a well-known source of *Amphioxus lanceolatus* (Pallas), and producing a macrofauna, dominated by lamellibranchs, long familiar to workers at Plymouth[1]. The position sampled is between 1 and 2 km. north-west of the Eddystone Rock, some 15 km. from the nearest mainland, and the depth of water above it is 23–28 fathoms.

This gravel also has given *Microhedyle lactea* and *Leptosynapta minuta* in small numbers. One oligo-

chæte worm (not yet determined) and five species of mites of the family Halacaridae have been isolated. Among these mites *Scaptognathus tridens* Trouess. and *Halacarus biculus* Viets are new to Britain according to Mr. G. O. Evans, of the British Museum (Natural History), to whom I am indebted for checking the identifications.

But the most notable feature of the Eddystone shell gravel is its rich fauna of small crustaceans, as first noticed by Prof. J. E. Harris during an unsuccessful search for young stages of *Amphioxus*. The smaller Malacostraca have been sorted from two separate major samples of gravel (using magnesium sulphate flotation technique), and so far 56 species have been listed. Of these some 20 are new to the Plymouth fauna[2] and five or six are new to science.

The many Amphipoda include a new species of *Metopa* and one of probably a new genus of Syrrhoidae, both among the more numerous species present in both major samples. (Two other new species of special interest are mentioned later.) Tanaidacea proved fairly numerous, dominated by *Typhlotanais microcheles* Sars and *Strongylurella indivisa* Hansen, both species new to Britain and rarely encountered anywhere. Among Isopoda, by far the most frequent have been *Paramunna bilobata* Sars and *Eurycope pygmaea* Sars, both well figured originally[3], but apparently the latter not previously known as British. Of special interest was the occurrence of a single example of *Microjaera anisopoda* Bocquet and Lévi[4], a particularly small blind janirid of 1·2 mm. described recently from two examples taken off Roscoff. Nothing else is known of this genus. More is known of another blind colourless genus, *Microcharon*, which is represented in the gravel by a new species, *M. harrisi* Spooner[5]. Six of the nine species of *Microcharon* so far discovered live in terrestrial ground-water in southern and eastern Europe, and two in coastal sand[6]. It is a genus belonging to the sub-family Microjanirinae, which is a typical element of the interstitial (phreatic) ground fauna, of which the bathynellids were the first to attract attention.

Two other blind 'interstitial' genera have now been found to be represented in the same shell gravel. One is a new genus of the amphipod family Bogidiellidae, so far represented by six species of *Bogidiella* from subterranean fresh water of coastal sand of southern Europe and Brazil. This is the first offshore occurrence of the family. The animal is very fragile, and only one of the fourteen specimens isolated is entire: this one shows a peculiar development of the seventh peræopod.

The remaining discovery is *Ingolfiella*, a genus which for long has stood as a separate sub-order of Amphipoda. Two specimens have so far been found, in a sample of gravel collected on March 30. The six species of this little-known genus show an extraordinary scattered distribution, ranging from the deep water of the North Atlantic (*I. abyssi* Hansen[7]) to caves in the Belgian Congo (*I. leleupi* Ruffo[8,9]). The newly found examples, the first from European seas, differ in small ways from four of the described species, but are clearly very close to *I. acherontis* Karaman from the ground-water of the Macedonian uplands (though presumably will not prove to be identical with it).

The occurrence of three phreatic genera in a marine deposit raises an interesting zoo-geographical issue. Each separately belongs to a taxon of at least sub-family status which is confined to the sub-surface domain, and has no normal (that is, eyed and pigmented) representative in the surface waters. These seem to belong to a slow-breeding (and slow spreading) faunistic element which is dispersed through the water held in the rocks and deposits below the lighted surface—not confined to continents, but continuously distributed both through them and under the sea bed. This view supposes that genera arise, disperse gradually in their native medium, and evolve, irrespective of whether air, fresh water, or ocean lie above the strata they inhabit. Any aquatic group, whether marine or fresh water, is a potential ancestor to members of such a fauna.

This idea will be followed up in future work, and the descriptions of new species mentioned above, it is hoped, published in the near future.

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Early Formation of Sperms in the *ey*^{2} Mutant of *Drosophila*

The testis of the newly hatched larva of wild-type *Drosophila* contains only spermatogonia which give rise to primary spermatocytes at the beginning of the second larval instar[1,2]. The primary spermatocytes which represent the early prophase of the first meiotic division have bigger nuclei and are larger in size and lie in the posterior end of the testis. This stage in the differentiation of the testis remains unchanged throughout the rest of the larval life. More advanced stages begin to appear after the formation of the puparium, so that 20-hr. old pupæ contain fully mature sperms.

The appearance of sperms in the larval testis has been reported as of rare incidence in the wild type[3]. On the other hand, sperm bundles showed up most frequently in the larval testis of some lethal mutants with prolonged larval life[4]. However, these usually disintegrate and give rise to patches of histolysed germ tissue.

In the present investigations, it has been found that the majority of *ey*^{2} late third instar larvæ, irrespective of the type of the eyeless phenotype which the larvæ will develop, contain stages up to and including sperm bundles. In some specimens, these advanced stages have become degenerate. 3-hr.-old eyeless prepupæ, without any exception, showed fully mature sperms. This shows that *ey*^{2} specimens reach sexual maturity at least 17 hr. before the normal time.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, June 15

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 3 p.m.—Annual General Meeting.

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. D. Bohm: "The Interpretation of Quantum Theory".

Wednesday, June 17

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. E. Knighting: "Numerical Experiments in the Integration of the Meteorological Equations of Motion"; Mr. J. S. Sawyer: "The Introduction of the Effects of Topography into Methods of Numerical Forecasting".

UNIVERSITY COLLEGE, DEPARTMENT OF PHILOSOPHY (in the Gustave Tuck Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. K. Ajdukiewicz (University of Warsaw): "What do Sentences State?"*

Wednesday, June 17—Saturday, June 27

INTERNATIONAL PLASTICS EXHIBITION (at Olympia, London).*

Thursday, June 18

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. F. C. Bawden, F.R.S.: "Viruses: Retrospect and Prospect" (Leeuwenhoek Lecture).

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. C. A. Rogers: "Coverings of n-Dimensional Space by Spheres".

ANTIQUARIAN HOROLOGICAL SOCIETY (at the Science Museum, Exhibition Road, South Kensington, London, S.W.7), at 7 p.m.—Mr. D. K. Packard (U.S.A.): "American Clocks 1780–1840".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—52nd Annual General Meeting. 8 p.m.—Prof. W. St. C. Symmers: "Clinico-pathological Observations on Some Cases of Fungal Infections and other Tropical Infections met with in Britain" (Series of lantern slides).

Friday, June 19

BRITISH INSTITUTE OF RADIOLOGY incorporated with the RÖNTGEN SOCIETY (in the Reid-Knox Hall, 32 Welbeck Street, London, W.1), at 11.30 a.m. and 2 p.m.—Radiobiology Meeting.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER IN PURE OR APPLIED MATHEMATICS—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (June 18).

ASSISTANT LECTURER IN PHYSICS—The Registrar, The University, Manchester (June 20).

ASSISTANT LECTURER IN THE DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY—The Dean, St. Thomas's Hospital Medical School, London, S.E.1 (June 20).

LECTURER or ASSISTANT LECTURER (preferably with a good honours degree in physics, together with research experience in some branch of biophysics or molecular biology) IN BIOPHYSICS—The Registrar, King's College (University of London), Strand, London, W.C.2 (June 20).

RESEARCH FELLOW IN BIOCHEMISTRY in the Department of Organic Chemistry, for work concerned with enzymic processes related to bacterial cell walls—The Registrar, King's College (University of Durham), Newcastle-upon-Tyne (June 20).

HEAD (man or woman with medical qualifications and an interest in education) OF THE DEPARTMENT OF HEALTH EDUCATION—The Clerk to the Governing Body, Battersea College of Technology, Battersea Park Road, London, S.W.11 (June 24).

LECTURER (Grade II) IN PSYCHOLOGY—The Registrar, The University, Bristol (June 24).

LECTURER IN PHYSICS—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (June 25).

LECTURER IN SOCIOLOGY—The Registrar, The University, Leicester (June 27).

ASSISTANT IN GEOLOGY, with curatorial duties in the Hunterian Museum—The Secretary of University Court, The University, Glasgow (June 30).

LECTURER IN THE HISTORY OF SCIENCE—The Registrar, The University, Leicester (June 30).

HEAD OF THE PLANT GENETICS SECTION—The Academic Secretary, Weizmann Institute of Science, Rehovoth, Israel (July 1).

RESEARCH STUDENTS (with the degree of Bachelor or Master of a British university other than Manchester) IN SCIENCE—The Registrar, The University, Manchester 13 (July 1).

LECTURER (qualified in chemistry, with teaching experience) IN EDUCATION—The Registrar, The University, Sheffield (July 4).

VICE-PRINCIPAL (graduate of a British university (any field), substantial teaching experience and some administrative and/or industrial experience)—The Principal, Reading Technical College, King's Road, Reading (July 6).

READER IN INDIAN PHILOSOPHY at the School of Oriental and African Studies—The Academic Registrar, University of London, Senate House, London, W.C.1 (July 8).

SENIOR LECTURER or LECTURER IN MATHEMATICS, and a SENIOR LECTURER or LECTURER IN CHEMISTRY (preferably Inorganic Chemistry) at University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 10).

LECTURERS or ASSISTANT LECTURERS IN ZOOLOGY at the University of Malaya (Singapore Division)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 15).

LECTURER/SENIOR LECTURER IN BIOCHEMISTRY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, July 18).

SENIOR LECTURER/LECTURER (with good general experience in the teaching of zoology and preferably a special interest in vertebrate zoology) IN ZOOLOGY at the University of New England, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, July 22).

LECTURER (preferably with a special knowledge of palæontology) IN GEOLOGY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, July 31).

LECTURER (with a higher degree with botany as the major subject) IN BOTANY at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, July 31).

LECTURER IN MATHEMATICS at Canberra University College, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, August 1).

SENIOR LECTURER IN PETROLOGY AND MINERALOGY at Canberra University College, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, August 1).

SENIOR LECTURER IN PHYSICS at Canberra University College, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, August 1).

CHAIRS (2) IN PHYSICS in the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, August 3).

CHAIR OF ZOOLOGY in the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand, August 31).

LECTURER IN PSYCHOLOGY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, August 31).

AGRICULTURAL OFFICER (TEACHING) (with a university degree in agriculture with farming experience and a diploma in education and/or teaching experience) in Uganda to teach general tropical agriculture to students taking a three-year course approximating to N.D.A. standard—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/9/028.

ASSISTANT LECTURER, Grade B (graduate, preferably with teaching or industrial experience) IN PHYSICS—The Principal, Medway College of Technology, Horsted, Maidstone Road, Chatham, Kent.

JUNIOR TECHNICAL OFFICER or TECHNICAL OFFICER (preferably aged 24–30, with H.N.C. in electronic engineering or equivalent qualifications, and preferably with experience of the electronic and development departments of the electronic instrument industry), to work on the design and development of electronic apparatus and instruments for use in medical research—Dr. F. D. Stott, Churchill Hospital, Headington, Oxford.

LECTURER, Grade B (graduate of a British university) IN SPECTROSCOPY—The Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

LECTURER (preferably with qualifications in zoology and teaching and/or research experience) IN BIOLOGY in the Training Department—The Warden, Goldsmiths' College (University of London), New Cross, London, S.E.14.

LECTURER (with an honours degree in physics and preferably some industrial experience) IN PHYSICS, to work mainly with National Certificate courses in Applied Physics and Engineering courses—The Principal, Cambridgeshire Technical College and School of Art, Collier Road, Cambridge.

MASTER TO TEACH PHYSICS—The Headmaster, Trinity School of John Whitgift, North End, Croydon.

ORGANIC CHEMIST (with a good honours degree in chemistry and postgraduate experience), to take charge of a small team concerned with the development and manufacture of compounds labelled with radioactive isotopes, in particular sulphur-35—The Personnel Officer, The Radiochemical Centre, U.K.A.E.A., Amersham, Bucks.

SENIOR LABORATORY ASSISTANT (preferably with experience in chemistry), to supervise the running of the new, well-equipped science laboratories—The Senior Science Master, Uppingham School, Uppingham, Rutland.

SENIOR TECHNICIAN (preferably with experience in biochemical or physiological techniques in man and animals), to take charge of the Medical Unit Laboratories under the Professor of Medicine—The Secretary, St. Mary's Hospital Medical School, Paddington, London, W.2.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 993, Vol. 251 (12 March, 1959): Cubic Forms in Thirty-two Variables. By Prof. H. Davenport. Pp. 193–232. 12s. 6d. No. 994, Vol. 251 (12 March 1959): Spherical Gravitational Waves. By W. B. Bonnor. Pp. 233–271. 12s. 6d. No. 995, Vol. 251 (12 March, 1959): Electrical and Thermal Resistivity

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